Analysis of hyperspectral images using spectral features calculated for blocks of pixels

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BOOK OF ABSTRACTS

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IASIM-14 Program

Wednesday December 3rd 2014

10.00 - 19.00 Registration
14.30 - 15.00 Opening
Session 1 – In memoriam of Jim Burger
15.00 - 15.30 Paul Geladi (SLU), "Reference standards: present and future"
15.30 - 16.00 Remembering Jim Burger: a colleague and a friend

16.00 - 16.30 Coffee Break + Poster Session

Session 2 – Chemometrics & Data Processing I
16.30 - 17.30 Plenary Lecture: Harald Martens (NTNU), "Measurement and modelling in six domains: (Time-, space- & composition) x (position & position change)"
17.30 - 17.55 Anna de Juan (University of Barcelona), "The multiset concept in image analysis: merging samples, spectroscopic techniques and algorithms"
17.55 - 18.20 Neal Gallagher (Eigenvector Research Inc.), "Synergy of target and anomaly detection in hyperspectral images"
18.20 - 18.40 Mario Li Vigni (University of Modena and Reggio Emilia), "Wavelet-enhanced multivariate image analysis for the food and materials sciences"

19.00 - 21.00 Welcoming Cocktail

Thursday December 4th 2014

08.30 - 13.00 Registration

Session 3 (Gordon-like) – Superresolution
09.00 - 09.50 Ludovic Duponchel (University of Lille), "The super-resolution concept: pushing the limits of hyperspectral spectroscopic imaging"
09.50 - 10.05 Discussant
10.05 - 10.30 Discussion from the floor

10.30 - 11.00 Coffee Break + Poster Session + Presentation by LOT-Quantum Design & Specim

Session 4 – Chemometrics & Data processing II
11.00 - 11.25 C. Ruckebusch (University of Lille), "A chemometric approach to single-molecule superresolution imaging. From spatial to spectral selectivity."
11.25 - 11.45 A. Ulrici (University of Modena and Reggio Emilia), "Exploration of datasets of hyperspectral images"
11.45 - 12.05 N. Gorretta (IRSTEA), "A spectral-spatial approach for hyperspectral image classification using spatial regularization on supervised score image"
12.05 - 12.25 S. Kucheryavskiy (University of Aalborg), "Analysis of hyper spectral images using spectral features calculated for blocks of pixels"

12.25 - 12.45 J.P. Wold (Nofima), "Strategies for multivariate regression and classification in on-line hyperspectral imaging systems"
12.45 - 13.00 A. Gowen (University College Dublin), "Hyperspectral image regression: model calibration and optimisation"

13.00 - 14.30 Lunch + Poster Session

Session 5 – Instruments and Remote Sensing
14.30 - 15.10 Keynote: Morgan L. Cable (JPL-NASA), "Understanding Worlds through 30 years of Spectral Imaging"
15.10 - 15.35 J.M. Carstensen (Danish Technical University), "Can reflectance spectral imaging and flourescence spectral imaging be effectively combined?"
15.35 - 16.00 S. Marshall (University of Strathclyde), "Folding PCA for feature extraction in remote sensing"
16.00 - 16.15 S. Blaaberg (Norsk Elektro Optikk AS), "The high-resolution integrated VNIR-SWIR airborne hyperspectral camera system HySpex ODIN-1024"
16.15 - 16.30 E. Guyot (Telops), "Airborne Midwave and Longwave Infrared Hyperspectral Imaging of Gases"

16.30 - 17.00 Coffee Break + Poster Session

Session 6 – Food & Agriculture
17.10 - 17.30 D. Airado-Rodriguez (Nofima), "Imaging photorreactions, chemical constinuents and sensory properties of complex intact samples via imaging of autofluorescence"
17.30 - 17.50 R. Calvini (University of Modena and Reggio Emilia), "Detection of contamination by aflatoxins on apricot kernels using NIR–hyperspectral imaging"
17.50 - 18.10 P.J. Williams (University of Stellenbosch), "Spectral imaging: a tool for maize grading"
18.10 - 18.25 P. Vermeulen (CRA-W), "Detection of plant contaminants in whole grains by NIR technology"
18.25 - 18.40 M. Corti (University of Milan), "The potential of imaging spectroscopy to estimate nitrogen and water content in maize leaves"

18.40-19.00 Software demonstration: PLS Toolbox + MIA (Neal Gallagher, Eigenvector Research)

20.30 Social Dinner at "Vecchia Osteria del Gelsomino" (Via del Gelsomino, 68 – Rome)

Friday December 5th 2014

Session 7 (Gordon-like) – Biomedical Applications
09.00 - 09.35 Neeta Sinnappah-Kang (Betty Cowan Research and Innovation Center), "*Fingerprinting* leukemic blood cells and multiantigen immunohistochemistry in brain tumor samples with chromogenic stains: plane scan spectral imaging in diagnostics"
09.35 - 10.10 Christoph Krafft (Leibniz Institute of Photonic Technology), "Overview and Perspectives on Raman and Infrared Spectroscopic Imaging for Brain Tumor Diagnosis"
10.10 - 10.30 Discussion from the floor

10.30 - 11.00 Coffee Break + Poster Session

Session 8 – Materials & Cultural Heritage

11.00 - 11.25 J. Linderholm (Umeå University), "Archaeology, near infrared spectroscopy and imaging combined with four levels of chemometrics"

11.25 - 11.45 J. Sandak (IVALSA), "Measuring wood with hyperspectral imaging"

11.45 - 12.00 I. Burud (NMBU), "Outdoor and indoor hyperspectral NIR imaging of wooden surfaces"

12.00 - 12.15 M. Calderisi (Archa), "Commercial black dyestuff supplier selection"

12.15 - 12.30 R. Palmieri (University of Rome La Sapienza), "Hyperspectral imaging applied to demolition waste: recycled products quality control"

12.30 - 12.45 C. Miliani (CNR-ISTM), "Chemical imaging of painting surfaces in the mid-IR range"

12.45 - 13.00 G. Capobianco (University of Rome La Sapienza), "Hyperspectral imaging applied to diagnostics and conservation: a methodological approach for pictorial layer characterization"

13:00 - 13.10 Closing

13.10 - 15.00 Lunch

POSTER LIST

P01 **Hyperspectral system based on two liquid crystal tuneable filters for early detection of citrus fruits decay**
D. Lorente, J. Gómez-Sanchis, N. Aleixos, S. Cubero, J. Blasco

P02 **Daily freshness decay of minimally processed apples using vis/nir multispectral imaging: preliminary tests**
R. Civelli, J.M. Amigo, V. Giovenzana, R. Beghi, R. Guidetti

P03 **Classification of Arabica and Robusta coffee samples subjected to different technological treatments using various image analysis methods**
R. Calvini, G. Foca, L. Bellucci, A. Ulrici

P04 **Analysis of baked sponges using hyperspectral imaging**
T. Kelman, P. Murray, S. Marshall

P05 **The use of hyperspectral imaging for predicting beef eating quality**
T. Qiao, J. Ren, C. Craigie, J. Zabalza, C. Maltin, S. Marshall

P06 **Hyperspectral imaging of *Danio rerio* (zebra fish): a preliminary study**
V. Olmos, L. Benítez, S. Piqueras, M.Casado, B. Piña, R.Tauler, A. de Juan

P07 **Hyperspectral imaging for the assessment of quality and safety of salmon and cod finfish**
C. Riccioli, E. Guzman, D.-W. Sun

P08 **NIR hyperspectral imaging and spectral band characterisation for agricultural products control**
D. Vincke, J.A. Fernández Pierna, P. Dardenne, V. Baeten

P09 **Identification of coccidiostats in feed additives by means of FT-NIR hyperspectral image analysis**
J. Omar, A. Boix, D. Vincke, J. A. Fernandez-Pierna, V. Baeten, C. von Holst

P10 **On field spectroscopy integrated techniques for biotic and abiotic stresses monitoring and mapping in precisione agriculture**
D. Masci, S. Mura, G.F. Greppi, L. De Cecco, S. Martini, F. Borfecchia

P11 **From laboratory to field scale hyperspectral imagery: platform setup and applications**
M. Moroni, A. Mei, E. Lupo, A. D'Andrea, F. La Marca, M.A. Boniforti

P12 **Inspection of log quality by hyperspectral imaging**
A. Zitek, F. Firtha, K. Böhm, V. Parrag, J. Sandak, B. Hinterstoisser

P13 **Mt. Etna (Italy) hyperspectral field database in support of multispectral and hyperspectral satellite data validation**
S. Amici, A. Piscini, M. Neri

P14 **Near infrared imaging spectroscopy on lithic materials from a mesolithic site in northern sweden**
C. Sciuto

P15 **Micro-Raman imaging for air particulate analysis**
R. Simonetti, M. Chöel, S. Lanteri, R. Leardi, L. Duponchel

P16 **Preparation of hyperspectral image data for online monitoring of crystallisation process of pharmaceutical compounds**
J. Dziewierz, S. Marshall, T.Kelman, A. Jawor-Baczyńska, J. Sefcik

P17 **Multitechnique image analysis: joining Raman and FT-IR biological tissue images with different spatial properties.**
S. Piqueras, M. Maeder, C. Beleites, C. Krafft, J. Popp, R. Tauler, A. de Juan

P18 **Ovarian cycle lipid dynamics revealed by hyperspectral DESI-MS imaging and multivariate data analysis**
V. Pirro, P. Oliveri, A.K. Jarmusch, C.R. Ferreira, R.G. Cooks

P19 **Infrared thermal imaging for use in restoration of defaced serial numbers**
I. Unobe, J.H. Kalivas, R. Rodriguez, A. Sorensen, L. Lau, J. Davis

P20 **Chemometrics revisited: hyperspectral imaging as a tool for investigating distributional hypotheses**
F. Marini

REFERENCE STANDARDS, PRESENT AND FUTURE

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Near Infrared Hyperspectral imaging instrumentation needs calibration for a number of parameters: geometry, focus, intensity and wavelength among others. The imaging instrumentation in Umea is presented and a number of primary and secondary standards studies are shown in more detail. The question is whether the present available materials are sufficient or whether new combination standards need to be developed. Standards and correct calibration were a major concern for Jim Burger, as can be deduced form reading his thesis, and the lecture will focus on standards work done or inspired by Jim.

MEASUREMENT AND MODELLING IN SIX DOMAINS: (TIME-, SPACE- & COMPOSITION) x (POSITION & POSITION CHANGE)

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New measuring opportunities give new research opportunities. But the explosive increase in good data creates a need for new data analyses. We need to extract the relevant and reliable patterns of information from the data and present it in a cognitively accessible form. Thereby, we can combine humble exploration of reality with proud utilization of humanity's established knowledge, i.e. combining Induction and Deduction.

Some sort of mathematical data modelling with statistical validation is then required. It is important to avoid both Macho-Modelling and Gucci-Statistics. ***Macho-Modelling*** is when we just force measured data into the straight-jacket of our favorite mechanistic model, whether it fits or not. ***Gucci-Statistics*** is when we adorn our publications with statistical p-values just to look good, academically. Multichannel spatio-temporal measurements deserve something better than that.

The world in which people live, - Midgard, the Norse called it - may perhaps be called *meso-cosmos*. It lies between the micro-cosmos of quantum mechanics and the macro-cosmos of relativity. Meso-cosmos is characterized by simple physics but complex chemistry, biology and culture. Here we can do people-relevant measurements in three "ontological" domains: in *time*, in *space* and with respect to *properties* like chemical composition. In each of these three fundamental domains, our measured signal intensity can tell about *position* and *position change*: The position itself shows how the measured intensity – conventionally plotted as ordinate or "y-axis", changes between samples. It may concern the intensity of a phenomenon measured at a given instrument channel (e.g. a wavelength position within color spectra), at a given point in time and e.g. in a given image position. The change in position, on the other hand, shows how the phenomenon is shifted along the abscissa or "x-axis" between samples. It may concern delays in time series, motion and deformation in images, or instrumental channel shift, e.g. in color wavelength.

Together we thus have *six ontological domains* that can affect our measured signal intensity. With modern instrumentation like hyperspectral video, MRI etc, we can measure intensity over time, over space (e.g. image pixels/voxels) and for many properties. From such data we can even differentiate between position and position-shift in each of the three domains. So, in principle, these six ontological domains of the world may be observed. The question is how to analyze such data.

Multivariate bilinear subspace models such as PCA, ICA, SNV, PLSR, L-PLSR, multivariate curve resolution etc, are useful for extracting and displaying essential structure within and between large data tables representing e.g. two domains. Techniques like EMSC can supplement these additive "series-expansion" models with non-additive elements. Cross-validation and other pragmatic assessments guard against overfitting. N-way extensions of the subspace models, such as Parafac, N-PLS and their nonlinear extensions, give efficient modelling of multi-way data tables that span e.g. three or more domains. Multivariate metamodelling helps us match mechanistic models and data.

But when the samples differ both along the "-y-axis" and along the "x-axis" at the same time, data modelling can become unpleasant: Just thinking about it can be confusing. Moreover, conventional subspace modelling is then inefficient. The present lecture concerns how to extend the subspace methods to the IDLE model, which may be used for combining all six domains. Examples from physiological metamodelling and medical video analysis will be used for illustration.

THE MULTISET CONCEPT IN IMAGE ANALYSIS: MERGING SAMPLES, SPECTROSCOPIC TECHNIQUES AND ALGORITHMS.

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The multiset concept in image analysis is extremely powerful to handle simultaneously several images coming from different samples and/or different imaging platforms and to combine data analysis tools oriented to provide complementary information about the systems analyzed [1].

A multiset structure is a big data table formed by pixel spectra from images of different samples recorded by the same technique and/or images of the same sample acquired by different spectroscopic platforms. To be able to buid a multiset structure, the spectral or the pixel mode of the images merged should be common. Thus, when the spectral mode is common, coupling images with different size, geometry and different spatial resolution is possible because the pixel direction can be completely different among images. When the pixel mode is shared, measurements obtained on the same sample with very diverse spectroscopic platforms can be combined and provide a much richer structural information about the image constituents. In the latter case, difficulties linked to different rotation/translation and spatial resolution among images should be surmounted to guarantee reliable results.
The wealth of information contained in an image multiset structure can only be fully exploited if treated with multiset analysis algorithms. In this sense, it is important that the full data analysis workflow keeps this concept in the application of all the involved data analysis tools (resolution/segmentation…).

All these concepts will be illustrated through single- or multistep multiset data analysis workflows of diverse multiset structures, formed by several images coming from different samples or techniques.

References:

[1] A.de Juan, S. Piqueras, M. Maeder, T. Hancewicz, L. Duponchel, R.Tauler, in: R.Salzer and H.W.Siesler (Eds.), Chemometric Tools for Image Analysis, in Infrared and Raman Spectroscopic Imaging, Wiley-VCH. 2014, pp. 57-110.

SYNERGY OF TARGET AND ANOMALY DETECTION IN HYPERSPECTRAL IMAGES

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Anomaly and target detection methods tend to be treated as independent algorithms for analysis of hyperspectral images. In general, target detection is a supervised pattern recognition technique while anomaly detection is unsupervised, exploratory analysis. Target detection algorithms such as generalized least squares (a.k.a. matched filter) typically employ known, well-characterized, library spectra for detection and classification. Unfortunately, due to measurement related differences between the library and measured data there may be mismatch between the library and measured spectra resulting in a compromise in detection performance. On the other hand, anomaly detection methods such as maximum signal factors (MSF) can be used with current measurements to explore anomalies where the observed anomaly is used as the measurement relevant target. Recent advances in MSF algorithms have improved detection performance, reduced memory and time requirements, and reduced the number of scores images that to be inspected during exploratory analysis. [1] However, the detected anomaly must be compared to library spectra for classification. As a result, it should be seen that target and anomaly detection form a complimentary approach to detection and classification resulting in measurement relevant target detection or "targeted anomaly detection". It is shown (Figure) how this approach can be used to a) tease out subtle target signal using whitening/weighting and b) optimize the preprocessing to be image specific.

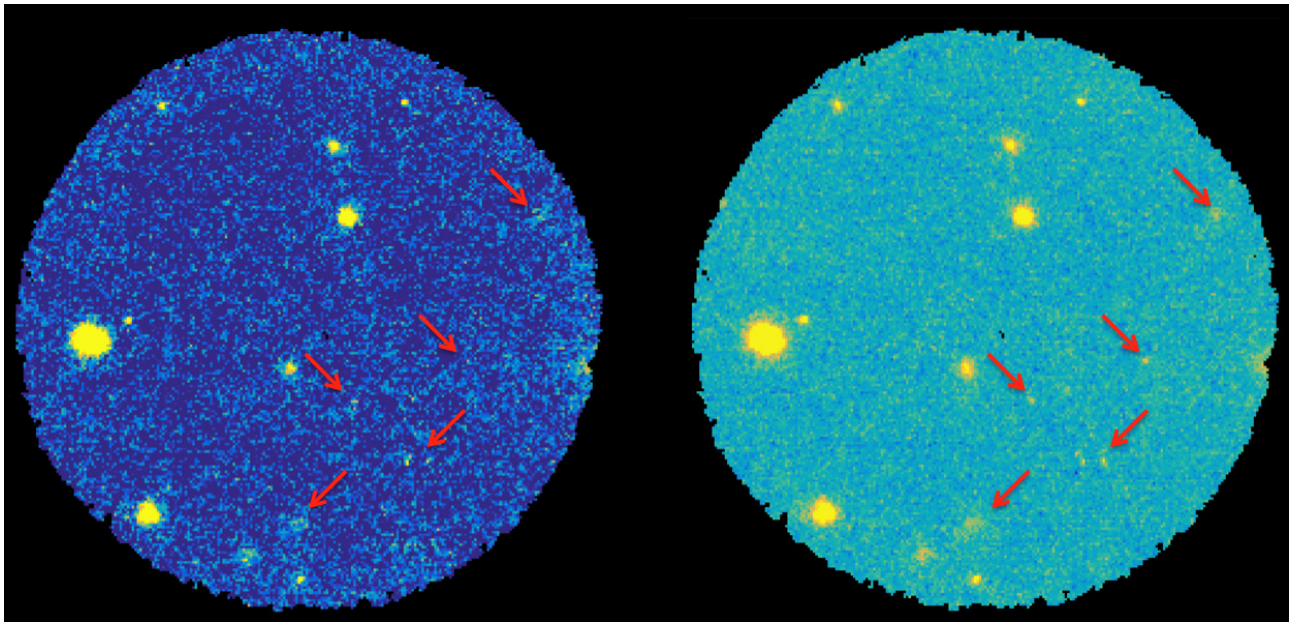

Figure. Comparison of GLS Target Detection (left) and MSF Targeted Anomaly Detection (right).

References:

[1] N.B. Gallagher, J.M. Shaver, R. Bishop, R.T. Roginski, B.M. Wise, *Decompositions with Maximum Signal Factors,* J. Chemometr., 28(8), 663, (2014), DOI: 10.1002/cem.2634.

WAVELET-ENHANCED MULTIVARIATE IMAGE ANALYSIS FOR THE FOOD AND MATERIALS SCIENCES

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Acquiring an image represents a powerful instrument to obtain, with a single measurement, information on the nature of a sample/system considering different aspects. This is particularly true when multi-channel images are used, where both compositional and texture information can be achieved in a single acquisition, but the image analysis advantage is still significant also when RGB or grey-scale images are acquired. Image analysis in Food and Materials sciences can give information on the texture and homogeneity of the sample, presenting a growing appeal for on-line monitoring of food products, for fast defects detection of defects. It can be also applied to microscopy images, to obtain information on a material both referred to its macro structure, and to its micro structure: local dominions of different physical and chemical nature can be highlighted and measured, in terms of dimensions, when working at pixel level.
Multivariate Image Analysis can be roughly summarised as the combination of two main steps of analysis: a phase in which the image is treated with a Feature Enhancement method, which highlights the correlation structure among features carried by the pixels, and a phase in which the obtained matrix is analysed by means of a Multivariate technique suitable for the problem. In this work, Wavelet Transform decomposition is used as a feature enhancement method alternative to Bharati and MacGregor's approach [1], with the two-fold target of highlighting the relationships among neighbor pixels and significantly decrease the dimensions of the data matrix [2]. Two wavelet decomposition schemes are proposed, in their 2D version: Discrete Wavelet Transform and Stationary Wavelet Transform, the latter having the advantage of maintaining the image dimensions throughout all the decomposition levels, thus facilitating the construction of the feature data matrix (no reconstruction step is required). Given a wavelet filter, the image is decomposed to a level, L, by using the 2D-DWT or 2D-SWT, applied separately to each color channel. Each decomposition block is either reconstructed to the original image dimensions or used as is, obtaining an image for each block and level: a total of 4 (Approximation, Horizontal, Vertical and Diagonal coefficients) times L (number of decomposition levels) times N (channels) images are obtained. The feature data matrix is the pixel-wise unfolded, variable-wise augmented concatenation of each of these blocks.
In the two proposed examples, different Multivarite Analysis tools are used. For the "Oranges dataset", where the target is detecting and recognizing which kind of defect is present on an orange surface, Principal Component Analysis is used to create class/defect sets from true images, and Partial Least Squares – Discriminant Analysis to build the model to evaluate new full-dimension images. For the "Nanoglasses dataset", gray-scale Transmission Electron Microscopy images of nano-structured TiO_2 glasses functionalised by the addition of Cerium, are analysed to evaluate the presence of dominions of different crystalline phases (at a ~ 2 nm scale), by using both Principal Component Analysis and Independent Component Analysis of the feature data matrix obtained from a 2D-SWT decomposition.

Acknowledgement: the TEM image analysed in the "Nanoglass dataset" has been provided by Gigliola Lusvardi and Gianluca Malavasi, Dept. of Chemical and Geological Sciences, University of Modena and Reggio Emilia. The "Orange dataset", together with part of the elaboration, was carried on in collaboration with Alberto Ferrer-Riquelme and José Manuel Prats-Montalban, Dep. de Estadística e IO Aplicadas y Calidad, Universidad Politécnica de Valencia.

References:
[1]. M.H. Bharati, J.F. MacGregor: Texture analysis of images using Principal Component Analysis. SPIE/Photonics Conference on Process Imaging for Automatic Control, Boston (2000) 27-37.
[2] J.M. Prats-Montalban, A. de Juan, A. Ferrer, Chemom. Intell. Lab. Sys., 2011, 107, 1.

THE SUPER-RESOLUTION CONCEPT: PUSHING THE LIMITS OF HYPERSPECTRAL SPECTROSCOPIC IMAGING.

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The increasing interest in nanoscience in many research fields like physics, chemistry, biology, including the environmental fate of the produced nano-objects, requires instrumental improvements to address the sub-micrometric analysis challenges. Hyperspectral imaging is a valuable tool to analyse complex and heterogeneous samples since it provides significant molecular information. The coupling of spectrometers and microscopes makes possible chemical map generation that represents the spatial distribution of chemical components within the sample. However, the spatial resolution limit with far-field spectrometer is first and foremost dictated by the photon wavelength due to diffraction limit. Considering vibrational spectroscopy, this drawback becomes a real constraint when micron or sub-micron sized samples are analysed. Two approaches have emerged to go beyond this limit. A first well-known approach focuses on the instrumental development such as near-field spectroscopy. A second approach, which will be introduced in this work, is an algorithmic one. Indeed the aim of super-resolution [1] consists of post-processing chemical maps in order to overcome the instrumental limits of imaging spectrometers. The proposed concept combines the information of a set of chemical images, slightly shifted from each other by a subpixel motion step, in order to generate results with a higher spatial resolution than that provided by the original measurements. After examining the theoretical basis of the super-resolution approach, we will demonstrate the possibility to go beyond the diffraction limit on 'target samples' corresponding to ideal and well-defined samples. Additionally, we will explore the potential of super-resolution applied to Near-infrared [4], Mid-infrared [3, 5] and Raman hyperspectral imaging [2, 6] for the analysis of real world cases.

References:

[1] S. Farsiu, M.D. Robinson, M. Elad, P. Milanfar Fast and robust multiframe super resolution. *IEEE Transactions on image processing*, 13(10), 1327-1344 (2004).
[2] L. Duponchel, P. Milanfar, C. Ruckebusch, J.-P. Huvenne, Super-resolution and Raman chemical imaging: From multiple low resolution images to a high resolution image, *Analytica Chimica Acta*, 607 (2), 168-175 (2008).
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[4] M. Offroy, Y. Roggo, L. Duponchel, Increasing the spatial resolution of near infrared chemical images (NIR-CI): The super-resolution paradigm applied to pharmaceutical products, *Chemometrics and Intelligent Laboratory Systems*, 117, 183-188 (2012).
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A CHEMOMETRIC APPROACH TO SINGLE-MOLECULE SUPERRESOLUTION IMAGING. FROM SPATIAL TO SPECTRAL SELECTIVITY.

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Single-molecule superresolution optical imaging relies on wide-field microscopy techniques that aim capturing images with a higher resolution than the diffraction limit. State of art methods are based on algorithms for localization of closely-spaced point-emitters or on statistical analysis of the temporal fluctuations of pixel signals.

In this work, we propose to investigate some chemometric approaches for single-molecule super-resolution data. We demonstrate that these methods can be useful for analyzing densely packed fluorophores in live-cell imaging. We also highlight the potential of penalized estimation using a L_0 norm penalty for the deconvolution of single-molecule superesolution imaging data. In both situations, contrast enhancement and improved spatial resolution can be achieved. Some examples are shown.

We also attempt to provide some perspective for bridging the gap from spatial resolution to spectral selectivity. Even though single-molecule superresolution imaging has become popular, simultaneous visualization of multiple molecular species in living cells remains difficult. It requires simultaneous imaging of several photoactivatable emitters, succesful signal detection, analysis and alignement of the digital images. A major breakthrough would be the capability to probe and unmix spectrally overlapping emmitters. This would bring chemometrics into play...

References:

[1] C. Ruckebusch, R. Bernex, F. Allegrini, M. Sliwa, P. Dedecker, J. Hofkens, t.b.s.
[2] J. De Rooi, C. Ruckebusch, P.H.C. Eilers, Anal. Chem. 2014, 13, 6291

EXPLORATION OF DATASETS OF HYPERSPECTRAL IMAGES

C. Ferrari, R. Calvini, G. Foca, A. Ulrici

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Hyperspectral images of size usually greater than 50 MB can be easily acquired in very short times, generally without the need of sample pretreatment. While Multivariate Image Analysis (MIA) tools can be efficiently used for the exploration of single hyperspectral images or of groups composed by a limited number (say up to 10) of merged images, the exploration of datasets composed by a large number (>10) of images is less straightforward. However, a representative sampling of a large number of specimens is frequently required to correctly estimate both intra- and inter-sample variability. This implies the acquisition of datasets composed by a large number of hyperspectral images and of several GB in size, especially in those cases where only one or a few samples can be included in a single image scene. In this context, the exploration of the dataset by applying MIA to single images or to subgroups of merged images does not allow to gain a global overview of the entire dataset variability and to properly highlight the possible presence of outliers, clusters and/or trends. A fast procedure which can be adopted to deal with this issue consists in computing the average spectrum of each image, to build a matrix of average spectra of the analyzed hyperspectral images. Although this approach leads sometimes to satisfactory results (especially when dealing with homogeneous materials), the information related to spatial variability is lost, and the hyperspectral image data are turned into "common" (i.e., not spatially resolved) spectral data. By averaging spectra, for example, the useful information related to the presence of a defect localized in a relatively narrow image area could be diluted within the massive amount of other "well behaving" pixels, becoming no longer detectable.

Aiming to develop a fast and easy-to-use tool able to facilitate the exploration of large datasets of hyperspectral images while maintaining both spectral- and spatial-related information of the original images, we have proposed an approach which consists in automatically converting each hyperspectral image into a signal named *hyperspectrogram* [1]. Essentially, the hyperspectrogram can be viewed as a fingerprint containing the relevant information brought by the original hyperspectral image, and is composed by a first part accounting for the spatial information and by a second part accounting for the spectral information. By representing each image with a vector of few hundreds of points, this procedure enables to compare simultaneously up to hundreds of images by means of common multivariate analysis methods, such as PCA.

In order to facilitate the exploration of datasets of hyperspectral images through hyperspectrograms, we have recently developed a Matlab Graphical User Interface (GUI), which easily allows calculation and visualization of hyperspectrograms, exploration of the dataset and visualization of the features of interest contained within each single sample directly in the original image domain.

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A SPECTRAL-SPATIAL APPROACH FOR HYPERSPECTRAL IMAGE CLASSIFICATION USING SPATIAL REGULARIZATION ON SUPERVISED SCORE IMAGE

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Introduction

The use of spatial constraints in the discrimination process of hyperspectral images is known as an effective way to increase the accuracy of classification. In this paper, we propose an new spectral-spatial discrimination approach which combines a supervised dimension approach and a spatial regularization process.

Methodology

The framework of the proposed approach includes three main step performed sequentially and described figure 1.

The first step deals with a supervised spectral dimension reduction approach (Partial Least Squares (PLS)) that transforms the hyperspectral image (IxJxP) into a score image (IxJxQ) that has a smaller number of channels (Q<<P). These channels are chosen so as to enhance differences between classes to be discriminated and reduce background variability, leading to edges that correspond to actual class borders.

In the second step, applying an edge preserving spatial regularization on this score image leads to a lowered background variability. We used the anisotropic diffusion method [1] to enhance the within region homogeneity while keeping intact the borders between adjacent regions. This method was originally developed for denoising gray-scale images by smoothing the image without removing the main edges. It consists of an iterative process in which, at each iteration, the amount of smoothing is weighted by the intensity of the local gradient value.

Therefore, in the third step, a pixel-wise classification of the regularized score image is applied. In this paper a k-nearest neighbor classifier was used.

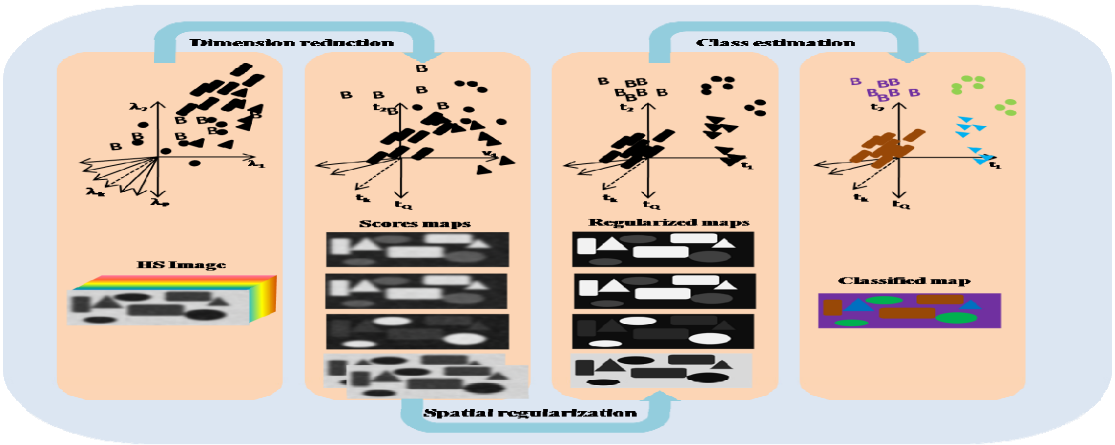

Figure 1 flowchart of the proposed approach

Results

The approach was tested on three hyperspectral images with different spatial and spectral resolutions. The classification results obtained with our approach were compared to those obtained with other spectral-spatial approaches already published and for a variable number of training samples. Obtained results show a real potential for the proposed approach.

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ANALYSIS OF HYPERSPECTRAL IMAGES USING SPECTRAL FEATURES CALCULATED FOR BLOCKS OF PIXELS

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In the recent years chemometric methods became quite popular also for exploring and analysis of digital images, especially multi- and hyperspectral, where number of channels can be very large and a lot of data has to be analysed. However in the most of the methods, image pixels are considered as separate objects without taking into account spatial relations among the pixels. Usually an image is unfolded into a matrix, where every row represents one pixel, so the image is treated just as a large set of spectra.

In the meantime, methods that utilise the spatial information are also in developing. One of them [1] was recently proposed for classification and discrimination of objects on hyperspectral images by calculating spectral features taking into account all pixels belonging to a particular object. In the present study the ideas from the proposed method were extended to solve a wider set of problems, including exploratory analysis and segmentation of hyperspectral images. Two approaches are suggested — calculation of spectral features for adjacent pixel blocks and for every pixel by considering the pixel's neighbourhood. Several simulated as well as real case examples will be shown to demonstrate the performance of the approaches.

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STRATEGIES FOR MULTIVARIATE REGRESSION AND CLASSIFICATION IN ON-LINE HYPERSPECTRAL IMAGING SYSTEMS

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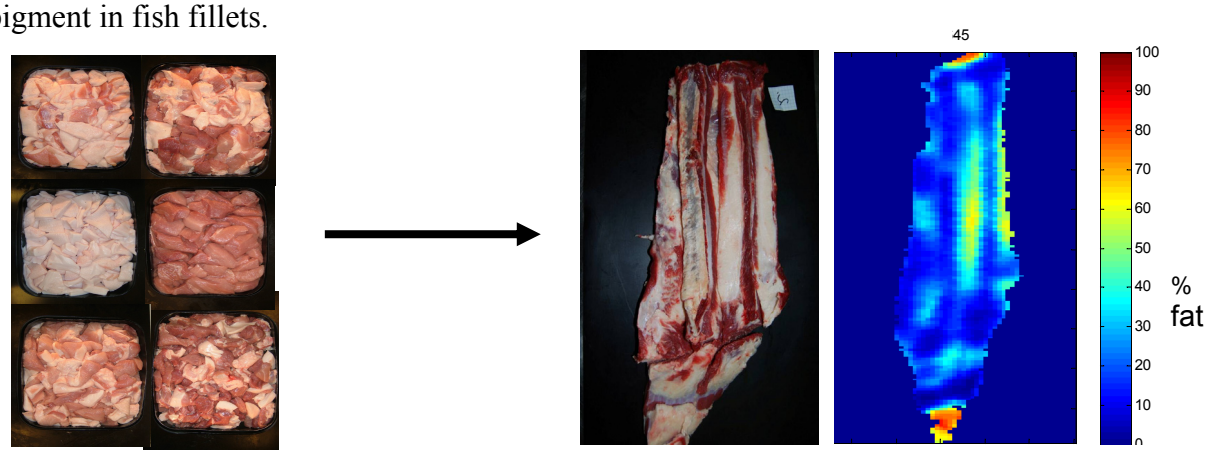
During the last 10 years Nofima has been developing on-line quality monitoring applications for the food industry based on hyper spectral imaging in the VIS-NIR region (460 – 1040 nm). The applications have been based mainly on a spectral flying beam system (TOMRA, Qvision 500); A beam scans across the conveyor belt at high frequency, spectra are collected at high speed and hyperspectral images of the material flow are obtained. The images are of relative low resolution spatially and spectrally, but more than sufficient for several important industrial food applications.

Regression models based on spectral features (X) and one or more reference analytes (Y) in hyper spectral imaging is not much different from multivariate calibrations based on regular two-dimensional spectral data. The main difference is the spatial dimension. The spatial dimension introduces rich opportunities for visualisation, but can also give challenges in proper reference analysis. In many cases, it is not feasible to conduct reference analysis on sample locations that can be matched with spectra from the same locations. Then it can be difficult to obtain calibration models that are reliable at pixel level. When working with foods – opposed to for instance human tissue -, we have rich opportunities to make for instance model systems that span the required variation in chemistry. Calibrations can be based on images and reference analysis of these model systems, and then be applied on complex real samples.

We have used three typical ways of quantitative regression calibration regimes:

1. Local image spectra (X) are modelled against corresponding local reference data (Y)
2. Average spectra (X) of rather homogeneous bulk samples are modelled against average reference values (Y) from the same samples using e.g. PLS regression. Regression vector is applied at pixel level for new and complex samples.
3. Only average reference values (Y) are obtainable for heterogeneous samples. Multivariate curve resolution (MCR) of X can be a solution and relate components to Y.

In this presentation it will be shown how these different strategies are used to make different food quality inspection applications. Most of the applications are in industrial use today. Some examples to be presented are on-line determination of fat and connective tissue in meat trimmings, fat determination and detection of discoloration and anomalous tissue in cod liver, and distribution of fat and pigment in fish fillets.



Calibration samples *Real sample* *Chemical image of sample*

Figure 1 From bulk sample model to detailed chemical imaging of real samples

HYPERSPECTRAL IMAGE REGRESSION: MODEL CALIBRATION AND OPTIMISATION

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Many different methods are available for the development of regression models from hyperspectral image data (e.g. partial least squares regression, principal components regression, ridge regression), all of which require the selection of samples to provide a representative spectra from which to develop calibrations against corresponding reference values (e.g. fat, moisture, protein content). This poses a problem in hyperspectral imaging: for most samples, it is practically impossible to measure the concentration of components at the pixel scale and therefore impossible to provide reference values for each pixel spectrum in a hypercube. To overcome this limitation, regression models are usually built using mean or median spectra computed over a spatial area of a sample, corresponding to a region for which a reference value is available. Models thus obtained can be applied to predict the composition of the individual pixel spectra in a hypercube. This results in a prediction map in which the spatial distribution of the predicted component(s) is visually interpretable. In this paper, it shown that the spatial distribution of components in prediction maps is influenced by numerous factors, including: spectral pre-processing methods, selection of calibration data and regression model parameters (e.g. number of latent variables in a PLSR model). Optimisation of these factors is crucial to prevent erroneous interpretation of prediction maps. The incorporation of spatial information in hyperspectral regression models is also discussed.

UNDERSTANDING WORLDS THROUGH 30 YEARS OF IMAGING SPECTROSCOPY

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Spectroscopy reveals physics, chemistry, biology and related processes. With advances in detectors, optics, and electronics, imaging spectroscopy became feasible in the late 20th century. Since its inception, the use of imaging spectroscopy on Earth and throughout the solar system has been proven and expanded extraordinarily. There are now a plethora of compelling science research examples for understanding worlds from the micron scale to exoplanet distances. The NASA Jet Propulsion Laboratory (JPL) has developed unique capabilities to enable the high fidelity instruments required to derive information of value from remotely measured spectra. Imaging spectroscopy enables remote measurement for the 21st century and beyond.

CAN REFLECTANCE SPECTRAL IMAGING AND FLOURESCENCE SPECTRAL IMAGING BE EFFECTIVELY COMBINED?

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For most applications spectral imaging will ideally create a traceable and reproducible reflectance spectrum in every pixel. From the reflectance image a lot of useful information about surface chemistry may be derived using multivariate statistical methods. Limitations in the quality of chemical information obtained are often set by the wavelength range covered by the acquisition system and the signal to noise ratio. By integrating spectral fluorescence imaging in a spectral reflectance imaging system we can obtain a significant improvement in the chemical information for many applications. Like reflectance imaging effectively maps chromogenic markers then fluorescence imaging maps fluorophores. Natural fluorescence (autofluorescence) from e.g chlorophylls, melanin, collagen, and a lot of microbial metabolites and spores makes it possible to improve the mapping and unmixing of these compounds.

We present a LED-based band-sequential imaging system for combined reflectance/fluorescence imaging, and discuss the steps involved in optimizing imaging, calibration, and analysis to explore the apparent potential of such a system. Applications from microbiology and plant systems will be used to exemplify the methodology.

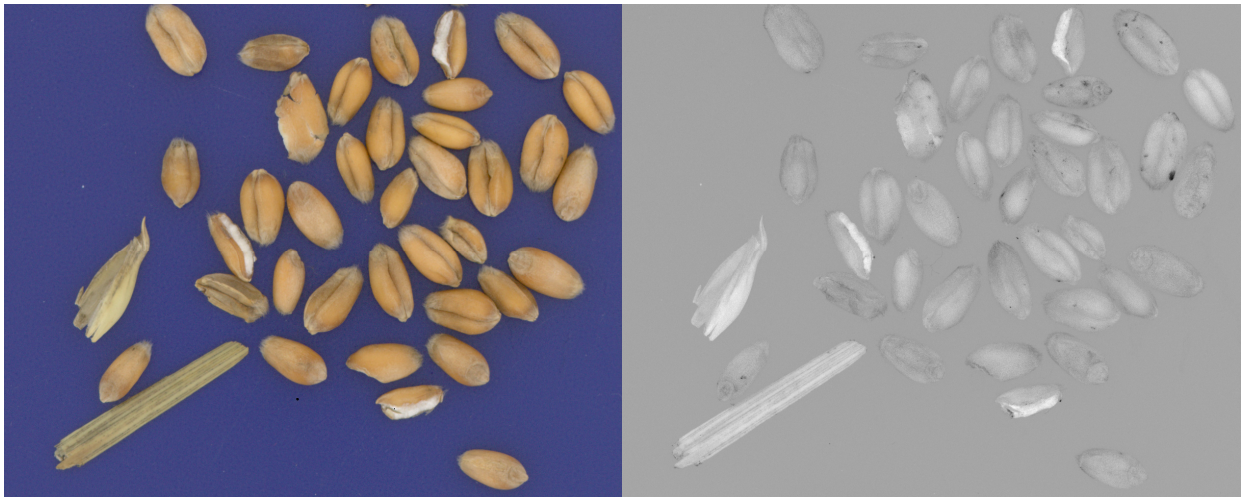

Figure 1 Wheat with low infection of bunt (left). Bunt spores visible in one fluorescence channel using spectral refelctance/fluorescence imaging (right).

Acknowledgement: This work has received partial funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 289108: The **TESTA project [1]**, and from the **Centre for Imaging Food Quality [2]** project which is funded by the Danish Council for Strategic Research (contract no 09-067039) within the Programme Commission on Health, Food and Welfare."

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FOLDING PCA FOR FEATURE EXTRACTION IN REMOTE SENSING

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Large dimensional datasets, such those in hyperspectral imaging (HSI), present great amount of information based on the numerous features available. The different bands contained in that spectral domain are in number of hundreds, which leads to high potential for discrimination capabilities. However, as the number of training samples is limited and the dimension of features in spectral bands is of hundreds, effective feature extraction is required and also feasible due to high redundancy between neighboring spectral bands.

Principal components analysis (PCA) is a common technique applied for feature extraction and reduction in correlated datasets [1]. However, when dealing with large dimensional datasets, conventional PCA analysis involves three main challenges. The first one is the computational cost demanded for large dimensional datasets, with high number of multiply accumulates (MACs) required. The second is related to memory handling, as data matrices become increasingly large arising difficulties to manage them. Finally, the third challenge is how to extract effectively the features as bands are equally treated when the covariance matrix is obtained but, however, not all of them provide the same useful information.

As part of our work, we propose an alternative approach namely Folded-PCA [2], in which the samples introduced to PCA analysis are transformed from vector to matrix form so the new matrix representation demands less computational cost while is able to extract more appropriate features, based on not only global but local structures, thanks to the folding process applied to the vector samples.

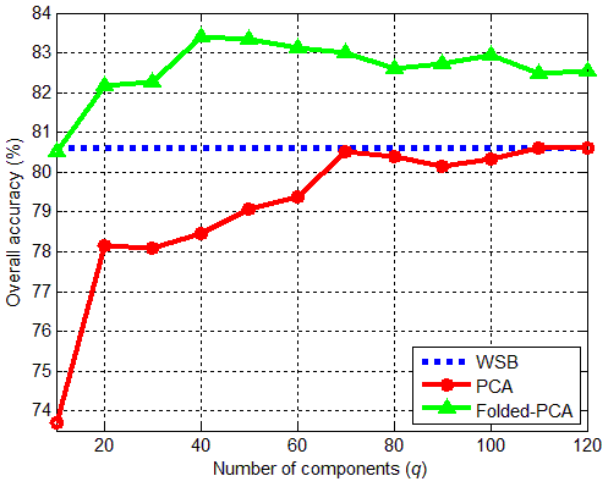

Figure 1 Overall Accuracy (%) in classification from WSB, PCA and Folded-PCA

Performance comparison based on HSI pixel classification using support vector machine (SVM) proves that our Folded-PCA is able to provide better classification accuracy than conventional PCA and even better than using original dimensionality (WSB in Fig. 1), with less computational cost.

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THE HIGH-RESOLUTION INTEGRATED VNIR-SWIR AIRBORNE HYPERSPECTRAL CAMERA SYSTEM HYSPEX ODIN-1024

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HySpex ODIN-1024 is a next generation airborne hyperspectral imaging system for the VNIR-SWIR spectral range developed by Norsk Elektro Optikk AS (NEO) [1]. Near perfect coregistration between VNIR and SWIR is achieved by employing a common fore-optics design. For the SWIR spectral range the across-the-track resolution of the instrument is 1024 pixels, while for the VNIR spectral range the user of the instrument can choose between a resolution of either 1024 or 2048 pixels. In addition to high resolution the optical design of the camera enables low smile- and keystone distortion as well as high sensitivity. In VNIR, resampling is utilized to correct for smile- and keystone distortion [2]. HySpex ODIN-1024 has integrated real-time processing functionalities for hyperspectral image processing and an onboard-calibration subsystem to monitor the stability of the system performance. HySpex ODIN-1024 has been developed within the French-Norwegian project SYSIPHE [3]. The partners in SYSIPHE are the French aerospace laboratory ONERA, the Norwegian Defence Research Establishment (FFI), and NEO. In addition to ODIN-1024 the SYSIPHE hyperspectral imaging system includes ONERA's hyperspectral imager SIELETERS, an instrument covering MWIR and LWIR. Together, ODIN-1024 and SIELETERS with a spatial resolution of more than 1000 pixels cover the atmospheric transmission bands over a broad spectral range going from visible to LWIR. FFI has developed a general software framework for real-time processing [4], which has been adapted for ODIN-1024 in collaboration between FFI and NEO. In addition, further real-time software was developed and real-time functionalities were implemented. We present an overview of the perpormance of the ODIN-1024 system including examples of data from airborne aquisistions.

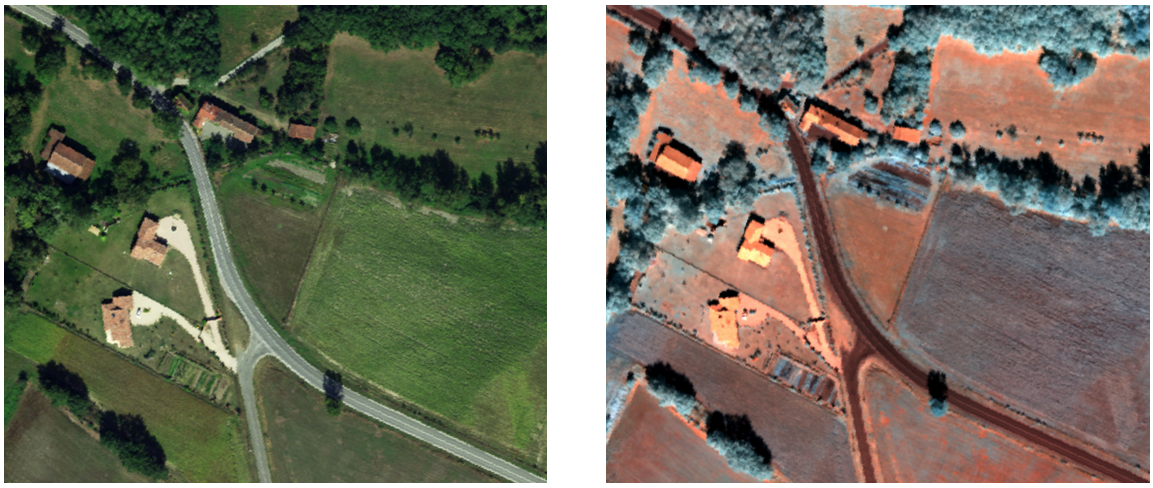

Figure 1 RGB representation of georeferenced and radiometrically calibrated image. VNIR (left), SWIR (right).

Figure 1 shows RGB representations of hyperspectral images aquired by HySpex ODIN-1024 in a flight line over Toulouse, France in September of 2013 during a SYSIPHE campaign.

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AIRBORNE MIDWAVE AND LONGWAVE INFRARED HYPERSPECTRAL IMAGING OF GASES

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Characterization of gas clouds are challenging situations to address due to the large and uneven distribution of these entities as a function of time. Whether gas characterization is carried out for gas leaks surveys or environmental monitoring purposes, explosives and/or toxic chemicals are often involved. In such situations, airborne measurements present distinct advantages over ground based-technics since large areas can be covered efficiently in addition to retrieving information from a safe distance. Airborne thermal hyperspectral imaging was carried out on smokestacks and a ground-based gas releases in order to illustrate the benefits of this technic to characterize gases. Quantitative airborne chemical images of carbon monoxide (CO) and ethylene (C_2H_4) were obtained from measurements carried out using a midwave (3-5 μm) and a longwave (8-12 μm) airborne infrared hyperspectral sensor respectively. Scattering effects were observed in the LWIR and MWIR experiments on smokestacks as a result of water condensation upon rapid cool down of the hot emission gases. Airborne measurements were carried out using both mapping and targeting acquisition modes. The later provides unique time-dependent information such as the gas cloud direction and velocity.

IMAGING PHOTORREACTIONS, CHEMICAL CONSTINUENTS AND SENSORY PROPERTIES OF COMPLEX INTACT SAMPLES *VIA* IMAGING OF AUTOFLUORESCENCE

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The potential of multispectral imaging of autofluorescence to map chemical components, photorreactions course and even sensory properties in intact complex samples is shown in this communication. Multivariate analysis is normally a must to interpret such complex images and extract relevant information and different strategies are presented here as well. The study of two very different case products is presented: photooxidation in cod caviar paste and naturally occurring components and oxidation development in mink skin. Cod caviar paste was stored over time, under different headspace gas composition and light exposure conditions, to obtain a relevant span in lipid oxidation and sensory properties. Front-face fluorescence emission images were obtained for excitation wavelength 382 nm at 11 different channels ranging from 400 to 700 nm (selected based on previous knowledge of the system [1]). We show how multivariate curve resolution (MCR) is able to extract and separate pure spectral components and predict their relative concentrations, which makes this approach a potent tool for investigation of the kinetics of autooxidation and photooxidation in complex intact biomaterials. Like an example, a chemical image representing the formation after light exposure of the main photoproduct of protoporphyrin IX which naturally occurs in cod caviar paste, is shown in Figure 1. We also show how it is possible to perform calibration *versus* sensory properties and further applying the obtained models for pixel-wise estimation of sensory flavors in real heterogeneous images, which gives rise for the first time, according to the related literature, to sensory images. Like another example of application it is presented how imaging of autofluorescence can map the distribution of collagen and elastin within mink skin and is also suitable to monitor and map the development of oxidation in this matrix, which is a real problem in the mink skin market.

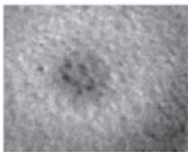

Figure 1 Drawing a photoreaction: predicted concentration of photoprotoporphyrin, the main photoproduct of protoporphyrin, after 14 days of light exposure of caviar through a pinhole.

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DETECTION OF CONTAMINATION BY AFLATOXINS ON APRICOT KERNELS USING NIR–HYPERSPECTRAL IMAGING

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Aflatoxins can be found as contaminants in a wide range of foods, such as nuts, cereals, dried fruits and milk. Due to their hepatotoxic and carcinogenic effects, the maximum allowed concentration of aflatoxins is nowadays regulated in many countries, with levels up to 50 µg/kg.
In routine analysis, the main methods used to determine aflatoxins are based on high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA) [1]. Despite the very high sensitivity of these methods, they are destructive, expensive, time consuming and not appropriate for real time control, e.g., online.
Consequently, the development of fast, non destructive and economic methods for aflatoxins detection and monitoring in food industry is becoming more and more important.
Our studies showed that manual sorting of dark or spotted apricot kernels removed 97.3-99.5% of total aflatoxins [2]. However, discolored seeds could be visually identified only after removing the skins from each seed by means of a time-consuming operation.
For these reasons, in this work we investigated the possibility to use NIR–HSI for the fast and non-destructive automated identification of aflatoxin contaminated unpeeled apricot kernels.
On the whole 9 hyperspectral images, each one containing 48 kernels, were acquired in the 900-1700 nm range. After image acquisition, the kernels were peeled to identify the dark or spotted kernels and subjected to HPLC analysis for AFB_1 quantification.
Classification models were then calculated on a training set of NIR spectra extracted from a representative number of non-contaminated and dark seeds, selected on 5 images on the basis of HPLC analysis results as well as of the visual evaluation of the peeled kernels. The remaining 4 images were instead used as independent test set for model validation. Since dark seeds were found to have a higher concentration of AFB_1 than spotted seeds, the latter ones were not included in the training set.
Different iPLS-DA classification models, built using different signal preprocessing methods and different interval size values, were then evaluated in terms of classification efficiency in cross validation of the training set pixels, in order to select the optimal conditions. The results were reported under the form of predicted probability maps, and for each single kernel the contamination was estimated as the percentage of pixels assigned to the "contaminated" class by the iPLS-DA model.

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SPECTRAL IMAGING: A TOOL FOR MAIZE GRADING

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Maize hardness is important to producers and processors in the grain trade since it greatly influences end-use processing performance. Maize has both glassy and floury endosperm within a single kernel in a particular ratio [1]. Hard kernels have predominantly glassy endosperm; soft kernels consist principally of floury endosperm while intermediate kernels possess approximately equal quantities of both. A number of techniques have been used to determine the hardness of maize [2]. A recent study compared different conventional techniques to predict dry-milling performance and showed that test weight was a good estimator of grain hardness [3]. However, all these methods were destructive. Previous work illustrated the potential of NIR hyperspectral imaging and multivariate data analysis for kernel hardness classification [4, 5]. The aim of this work was to develop NIR hyperspectral imaging into a tool for maize grading with particular focus on kernel hardness for use in the maize industry in South Africa.

NIR hyperspectral images of commercial maize hybrids with known hardness were collected using an UmBio Inspector (UmBio AB, Umeå, Sweden) in the wavelength range 1000-2500 nm. Based on kernel composition and using the brushing technique, the data was divided into two groups, *i.e.* hard and soft endosperm. Using this information, PLS-DA models were calibrated and validated on a limited set of kernels to classify endosperm type and thus predict kernel hardness. A PLS-DA model with 2 factors was calculated for an R^2 of 81%. The validation results indicated that the model was capable of classifying endosperm hardness with high accuracy and a low error (RMSEP 0.23), Table 1. The model correctly predicted 96.8% of the pixels belonging to the hard endosperm group and 98.2% of the pixels belonging to the soft endosperm group. A small number of pixels were incorrectly predicted as soft (3.21%) and hard (1.75%), respectively. This model was further used to classify endosperm hardness in a number of images, not included in initial model development, with high accuracy.

Table 1. Validation of PLS-DA model for prediction of endosperm hardness in commercial maize kernel hybrids

Groups:	Number of pixels in each group	Number of pixels predicted as Hard Endosperm	Number of pixels predicted as Soft Endosperm
Hard Endosperm	27246 (54.5%)	26371 (96.8%)	875 (3.21%)
Soft Endosperm	22683 (45.4%)	397 (1.75%)	22286 (98.2%)
Not Classified	50 (0.1%)	50 (100%)	
Total	49979 (100%)	26818 (53.7%)	23161 (46.3%)

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DETECTION OF PLANT CONTAMINANTS IN WHOLE GRAINS BY NIR TECHNOLOGY

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Introduction

Since decades, near infrared (NIR) spectroscopy is widely used in the food and feed chains to implement rapid, inexpensive and efficient control tools to generate a fingerprint of the products for their quality control. This fingerprint can be altered by several factors as the presence of undesirable substances, which will modify the pattern of the spectrum. It was shown that a classical NIR spectrometer equipped with an adequate specific sample presentation allowed analysing a larger number of reduced size subsamples by acquiring one spectrum by subsample. That was demonstrated through a study based on the detection of *Datura stramonium* seeds in buckwheat grains. This plant contaminant is a wild-growing plant found in crops and known for its high content in alkaloids. Chemometric tools such as PCA, applied to the NIR spectra allowed discriminating between pure Datura seeds, buckwheat kernels and mixtures of both. In a similar way, the aim of this work is to demonstrate, through one case study on the detection of ergot bodies in cereals, the performance of NIR technique when a spatial dimension is used through the use of hyperspectral imaging.

Results and Discussion

In this study case, the on-line detection is investigated using NIR hyperspectral imaging, which combines spectral and spatial information. Up to several thousand spectra of single kernels per sample can be collected. This study aims to assess in cereals, the use of NIR hyperspectral imaging to detect and quantify ergot bodies rich in toxic alkaloïds, formed by the fungi *Claviceps purpurea* [1,2]. It was shown that, using NIR imaging system, the individual elements of up to 50 kg of cereals can be analysed in 1 hour. The LOD of the method has been determined at 145 mg/kg, well below the limit of 500 mg/kg fixed by the European Commission in "intervention" cereals destined for humans.

Conclusion

The high throughput sampling accessories and the hyperspectral imaging technology are adequate tools to bring the NIR spectroscopy in the field of the detection and quantification of contaminants. They allow scanning rapidly small sample fractions in order to lower the limit of detection of the NIR techniques and to match the official control requirements. This approach can be used as control tool at the reception of grains.

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THE POTENTIAL OF IMAGING SPECTROSCOPY TO ESTIMATE NITROGEN AND WATER CONTENT IN MAIZE LEAVES

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Optical sensing can be useful for agricultural applications because it is, if compared to laboratory analysis, a non-destructive and quick technique that can be used to drive site-specific irrigation and nitrogen fertilization, allowing to preserve optimum yields while improving the environmental performances of agricultural systems. Tractor-mounted multispectral sensors are already used to drive on-the go nitrogen fertilization and multispectral sensors e.g., digital cameras mounted on unmanned aerial vehicle (UAV), are studied at the level of technology demonstration because they can be used to make field maps for precision agriculture. Between optical sensors the hyperspectral ones are of increasing interest because of their high throughput and lowering prices. In particular, imaging spectroscopy is suited for precision agriculture because it contains spatial information about the distribution of the reflectance properties of the crop, but still a few studies were made about its potential.

A preliminary experiment at laboratory scale was made by scanning maize leaves. The leaves were selected from an experimental field on nitrogen fertilization using digested manure with six theses of nitrogen fertilization. Hyperspectral images of three slices (bottom, medium and tip) per leaf were collected with a hyperspectral imaging system working between 512 and 1768 nm. Two circles (diameter of 7 mm) per scanned leaf slice were collected after scanning for reference water and nitrogen content determination. The dataset of 57 hyperspectral images was used to construct two prediction models, to predict nitrogen content and water content using the PLS regression method, and the use of mean and median spectrum of the sampled ROIs was compared. Nitrogen model resulted to have a RMSECV of 0.16% (RPD=2.1) and water content of 2.14% (RPD=2.2), R^2 were 0.78 and 0.71, respectively. Two common multispectral vegetation indices, the Normalized Difference Vegetation Index (NDVI) and the Photochemical Reflectance Index (PRI) were also extracted from the spectral data and gave worse prediction results with respect to PLS modeling for nitrogen and water content prediction. It was also investigated the penetration depth achievable with hyperspectral imaging (figure 1) and it was found that the signal can reach a depth between three and five leaf layers, showing the possibility of retrieving information on whole crop canopies and estimation of the LAI (Leaf Area Index) at least at the beginning of crop growth; the effect of a scattering and non-scattering background on leaf reflectance was also investigated. These findings have important practical consequences on the use of these instruments in field.

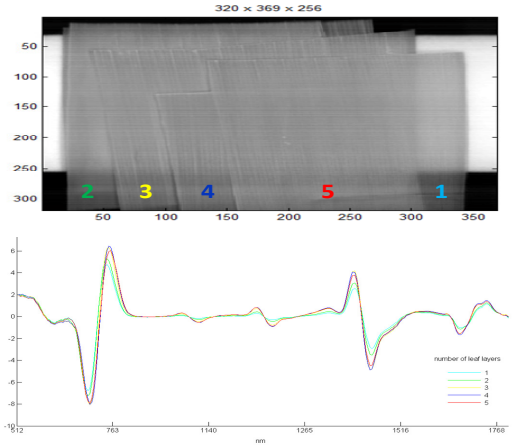

Figure 1. Hyprespectral image of overlapping corn leaf slices (from 1 to 5 layers) and second derivative spectra extracted from ROIs with different overlapping degrees.

[*] James passed away this past August. The authors are very saddened by the loss.

‘FINGERPRINTING’ LEUKEMIC BLOOD CELLS AND MULTIANTIGEN IMMUNOHISTOCHEMISTRY IN BRAIN TUMOR SAMPLES WITH CHROMOGENIC STAINS: PLANE SCAN SPECTRAL IMAGING IN DIAGNOSTICS

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Spectral images contain an abundance of data but potent use of the data entails an understanding of the character and shortcomings of the data including diverse strategies for processing and elucidating it.
We conducted a pilot study on ‘fingerprinting’ leukemic cells using plane scan hyperspectral imaging. By creating a spectral library for the different cells, we found that the subtle biochemical changes (within the cells) reflected by the way the staining dye binds in ‘healthy’ versus ‘cancerous’ cells were powerful in discriminating these cells, even between different leukemia subtypes (Figure 1).
By using the chromogenic-based staining approach (for brightfield imaging), we investigated the potential of spectral unmixing of multiantigen immunohistochemistry labeling on a single slide. We discuss data generated on combining 3 markers that localized in the apical membrane, cytoplasm and nucleus/cytoplasm, respectively (Figure 2). The imaging system allowed even the spectral unmixing of co-localized proteins of interest. In order to obtain quantitative data on the number of immunopositive cells, the tissues were counterstained with hematoxylin. The method was sensitive in detecting low levels of proteins, even when the signal was ‘covered’ by a more dominant color and may not be visualized by the naked eye. The procedure allowed the use of higher dilutions of primary antibodies, which cut costs for processing the slides. As the final result of the ‘4-color’ staining needed to be ‘transparent’, it decreased time required to stain the slides. The most crucial part of using the spectral imaging technology was the generation of a spectral profile for each chromogenic color. The colors have to be a non-scatterer, following the Beer-Lambert’s law [1].

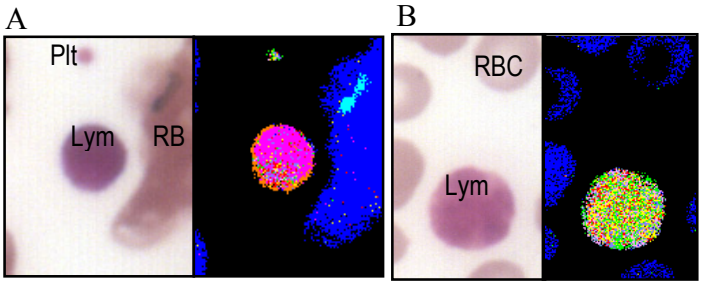

Figure 1 RGB (L) and hyperspectral (R) images of a lymphocyte from the blood smear of a healthy volunteer (A) and CML (B) patient. [Lym: Lymphocyte; Plt: Platelet; BRC: Red blood cell]

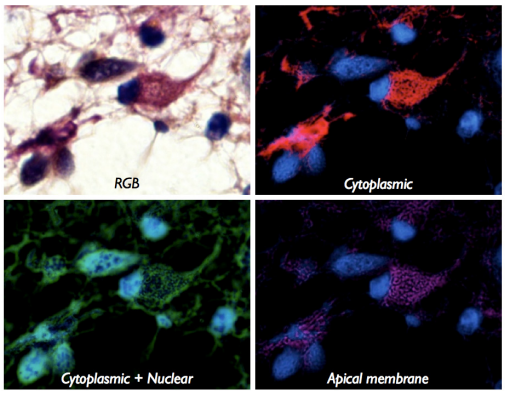

Figure 2 Spectral-based unmixing of triple antigen stained brain tumor tissue counterstained with hematoxylin.

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OVERVIEW AND PERSPECTIVES ON RAMAN AND INFRARED SPECTROSCOPIC IMAGING FOR BRAIN TUMOR DIAGNOSIS

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Raman and infrared spectroscopy probe molecular vibrations. Combination with lateral information gives hyperspectral imaging data that constitute powerful a biomedical approach to complement standard clinical methods. An overview is given for technical implementations and the application of vibrational spectroscopy to assess primary and secondary brain tumors. In general, these techniques can be transferred to other pathologies, in particular in the context of lipid-related pathologies such as atherosclerotic plaques and non-alcoholic fatty liver.

Fourier transform infrared (FTIR) imaging combines the multiplex advantage of interferometers and the multichannel advantage of focal plane array detectors and enables parallel registration within the field of view typically between 300×300 and 700×700 μm^2. Raman images are usually collected in the serial registration mode scanning the laser focus across the specimen. Near infrared excitation lasers (e.g. 785 nm) offer advantages of reduced autofluorescence background and enhanced penetration.

FTIR imaging was applied to determine the tumor grade of gliomas [1] – the most frequent primary brain tumors – and identify the origin of brain metastases [2, 3] – secondary brain tumors that are more frequent than primary brain tumors. A key parameter to distinguish normal brain tissue from tumors is the lipid to protein ratio. The strategy to identify the primary site is based on organ-tissue signatures that can be detected by IR and Raman spectroscopy. Whereas FTIR imaging is advantageous for rapid molecular characterization of dried tissue sections, Raman microscopy offer advantages for non-dried specimens, the spatial resolution is superior and fiber optic probes can be applied in vivo. Raman images classified brain metastases with similar accuracy [4]. Chemical features in Raman spectra such as the content of proteins, lipids and nucleic acids were shown to correlate with morphological properties such as the number and size of cell nuclei [5]. First in vivo Raman studies were reported for a mouse model with brain metastases. After inserting a cranial window, fiber optic Raman imaging identified the tumor margins [6].

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ARCHAEOLOGY, NEAR INFRARED SPECTROSCOPY AND IMAGING COMBINED WITH FOUR LEVELS OF CHEMOMETRICS

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Archaeological research is, as in many sciences, cumbersome and time-consuming. Representative sampling of artefacts or soils samples for laboratory wet chemical analysis is quasi impossible. Solutions are found in fast portable near infrared measurement or in hyperspectral scanning of large areas. With hyperspectral imaging, which is the fastest method, an NIR spectrum is available for each pixel in the scan. For portable NIR measurement, "pixels" are created by scanning in a regular grid.

In all the above cases, huge data sets are available for analysis by chemometrical methods. Exploratory chemometrics (level 1) gives a contrast between normal and outlier samples, where the outliers may be the most interesting parts. Level 2 is regular classification (supervised or unsupervised). Level 3 is regression against an external parameter combined with prediction in each pixel to produce a prediction map. Level 4 is the hardest: it consists of chemical, spectroscopic and physical interpretation based on the conclusions of the other 3 levels. And to this one has to add to archaeological interpretation.

Examples from soil analysis, artefact analysis and the analysis of rock painting will be presented to illustrate this.

MEASURING WOOD WITH HYPERSPECTRAL IMAGING

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Wood is a natural material widely used in numerous applications, including construction, furniture, paper, bio-energy and others. From the chemical point of view, wood is a complex matrix of several polymers, where the most important ones are lignin, cellulose, hemicelluloses, extractives and minerals. The spatial distribution of such chemicals is very complex due to wood heterogeneity and anisotropy, as well as anatomy and physiology of the living tree from which wood has been extracted. Wood is a highly hygroscopic material, responding to the humidity changes of surrounding air. The variability of wood properties is very high at the level of one wood species, same forest, single tree, or even within one piece of wood. Such properties are, however, extremely important for a proper use of wood. Early detection of the superior wood characteristics, as well as identification of any defects, may significantly influence the economical balance of the whole forest-wood chain as well as may contribute to sustainable use of natural resources. A lot of research efforts have been directed toward identification of novel methods capable of assisting quality sorting. It was shown that hyperspectral imaging is a very promising alternative. Some preliminary research has been already reported [1,2], however, real life applications toward forest/timber industry are still under development. Project SLOPE is one of such initiatives, where ten partners from Europe were collaborating with BurgerMetrix in application of hyperpsectral imaging (among others) into quality grading of wood during harvesting operations. The preliminary results on the laboratory-conditioned samples are presented below (Figure 1). The hyperspectral image of wood (average reflectance of spectrum in the whole near infrared range) is highly affected by the surface roughness. However, despite apparently worst quality, the rough surface images are still suitable for detection of wood defects.

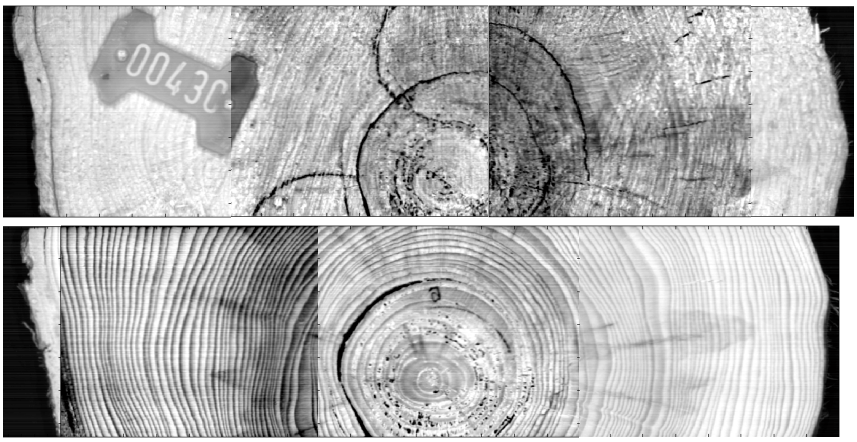

Figure 1 Hyperspectral images of cross section of log containing various wood defects (Note: the upper image measured on rough surface and bottom the same log but smooth surfaces)

Acknowledgement: We would like to dedicate this presentation to the memory of Dr James Burger.
Part of this research has been conducted within frame of the SLOPE project (FP7- Collaborative Project – 604129). The project SLOPE has received founding from the EU, and it has been co-founded as part of the 7th Framework Programme. Authors are solely responsible this work does not represent the opinion of the EC. The EC is not responsible for any use that might be made of information contained in this paper.

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OUTDOOR AND INDOOR HYPERSPECTRAL NIR IMAGING OF WOODEN SURFACES

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Hyperspectral near infrared imaging has been applied in an on going field study of fungal growth on a variety of wood substrates exposed in an outdoor environment, as a follow up to a laboratory hyperspectral study of fungi on wood surfaces [1]. Five different wood substrates were exposed vertically facing north and south in Ås, Norway, in a specially designed test set-up. A 3m long translation stage was mounted in a fixed position 100 cm from the samples to ensured that the samples were scanned by the hyperspectral camera at the same distance each time. Hyperspectral measurements were carried out both outside and inside in order to explore the influence of the different light conditions. For the outdoor measurements, only natural sunlight was used and the spectra therefore have gaps due to water vapour absorption in the air. For the indoor measurements the samples were brought temporary inside and scanned with artificial light.
Segmentation of the mould growth on the wood surfaces was carried using Spectral Angle Mapper (SAM [2]) by picking mould reference spectra from one of the samples. The SAM based segmentation works equally well on the outdoor measurement as for the indoor measurement, indicating that the varying sunlight and atmospheric absorption is not a problem for this specific application. An example of SAM segmentation on the Aspen wood substrate is shown in Fig. 1 (left and middle panel). Growth curves of the fungal growth could therefore be obtained for all the samples on the outdoor measurements (Fig. 1 right panel). There are however, several challenges for these types of studies. On surfaces from several wood substrates there are structures from growth rings, knots and sometimes cracks. These will both influence the spectra from the wood and also the fungal growth on them. Moreover, the wood substrates exposed in an outdoor environment will have a fungal surface growth caused by a mixture in species of mould and blue stain fungi, with somehow different spectra. Finally, the fungi will be in different phases of growth, and previous laboratory measurements indicate that this also yield different spectral behaviour. These challenges and ways to overcome them will be discussed.

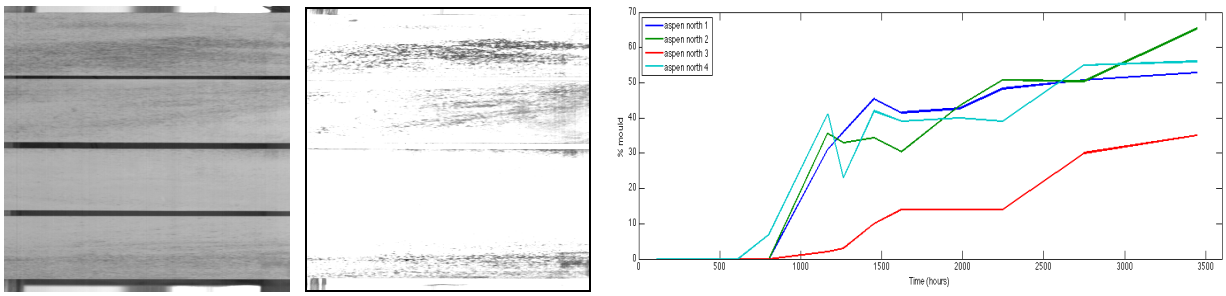

Figure 1 Left : Outdoor image of Aspen wood samples, from one of the bands in the hyperspectral image cube. Middle : Segmentation of fungi on the Aspen surfaces using SAM on the outdoor measurements. Right : Growth curve of the four Aspen samples from August 2013 until January 2014.

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COMMERCIAL BLACK DYESTUFF SUPPLIER SELECTION

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The aim of this work was to define an analytical procedure to select the most performing black dye, in term of color yield on leather, among different batches derived from six different suppliers, as a decisional tool to support the tanning company. This type of azo dye, Acid Black 210, is widely used commercially in the leather sector. The manufacturing of azo dyes is based on batch processes. Therefore, according to the different processes used, the final product may present different features in terms of composition and colour yield; so, the virtually "same product" can be different from different producers and in different batches supplied by the same producer. For this reason, it is necessary to provide the leather company a procedure that allows selecting the producer with the most performing product, in order to guarantee the control of the quality of the final coloured leather. The dye batches coming from six different suppliers were named A, B, C, D, E, F, respectively. Five samples from each supplier were examined, determining main inorganic anions and cations, collecting FT-ATR spectra on neat samples and their ashes, analysing UV-VIS spectra in water solutions. In addition the colourgrams of leather samples coloured using the same process recipe, were acquired [1]. PCA analysis was carried out on each dataset. Ion analysis (revealing which salts are used for blending) showed that the dye from the three producers A, B and C, is richer in $SO4^{=}$, the dye from D, E and F is richer in Cl^{-}; furthermore, dye from F contains more $CO_3^{=}$ than the others. In addition, UV-VIS spectra on the water solution buffered at pH=7.9 help in distinguishing between different suppliers (score plot in Figure 1). Colourgrams analysis showed some interesting features. Blackness score (lower RGB values and high "Intensity"), summarized on PC1,was higher with the E dye, and lower with the A dye, whose leather samples appeared more uniformly coloured but less "black" (Figure 2). The chemical composition seemed to markedly affect leather colour, for dye containing sulphates (top left of the score plot). Hue is explained along the PC2. Samples on the upper part of the scores plot tend to have a yellowish shade, while sample on the opposite side a reddish one. D and F samples have the most homogeneous hue, the former tending to yellow, the last to red. In conclusion: ion, UV-VIS at pH ~ 8 and colourgrams analyses is the set of tests to assess the dyeing yield on leather. According the results obtained, the dye from producer D showed the highest dyeing yield. This result was confirmed by the tanning company specialists.

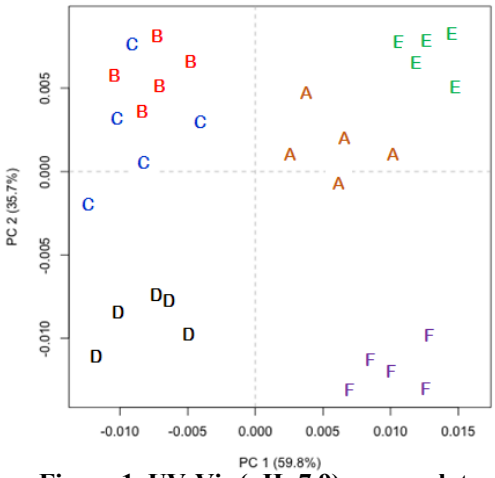

Figure 1. UV-Vis (pH=7.9) scores plot

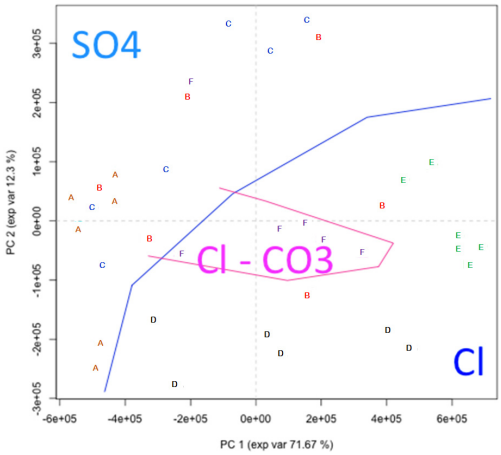

Figure 2. Colourgrams scores plot

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HYPERSPECTRAL IMAGING APPLIED TO DEMOLITION WASTE: RECYCLED PRODUCTS QUALITY CONTROL

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The development of innovative construction and demolition (C&D) waste recycling technology in order to create high-value "green concrete" and the replacement of primary raw materials through **E***nd*-**O***f*-**L***ife* (EOL) concrete are two of the most important problems to face and solve in the secondary raw materials sector. The possibility to realize, implement and set-up efficient sorting and/or quality control strategies for the collection of information concerning C&D waste materials, plays an important role to perform non-destructive, rapid and low cost analyses finalized to preliminary detect and characterize the materials constituting C&D waste stream. The main aim of this study is a C&D waste characterization adopting an **H***yper***S***pectral* **I***maging* (HSI) based approach. This technique was specifically applied in order to recognize concrete aggregates and other materials considered as contaminants. The developed procedures are based on the utilization of a laboratory device working in the near infrared range (1000-1700 nm): NIR Spectral Camera™, embedding an ImSpector™ N17E (SPECIM Ltd, Finland). Acquired hyperspectral images were analyzed adopting the PLS_Toolbox (Version 7.8, Eigenvector Research, Inc.) running into the Matlab® environment (Version 7.11.1, The Mathworks, Inc.). Different chemometric methods were applied: ***P****rincipal* ***C****omponent* ***A****nalysis* (PCA) for exploratory data approach and ***P****artial* ***L****east* ***S****quare-* ***D****iscriminant* ***A****nalysis* (PLS-DA) to build classification models. Starting from a reference 'training image set', spectra representative of materials constituting the C&D waste stream have been preliminary collected (Figure 1) in order to define the classification algorithm. The developed classification procedure was then validated using external datasets. Results showed as, with the proposed approach, recycled aggregates can be identified and distinguished from contaminants (e.g. bricks, gypsum, plastics, wood, foam, etc.). The developed procedure based on HSI in the NIR range can be applied for quality control of recycled aggregates to be used in new concrete production.

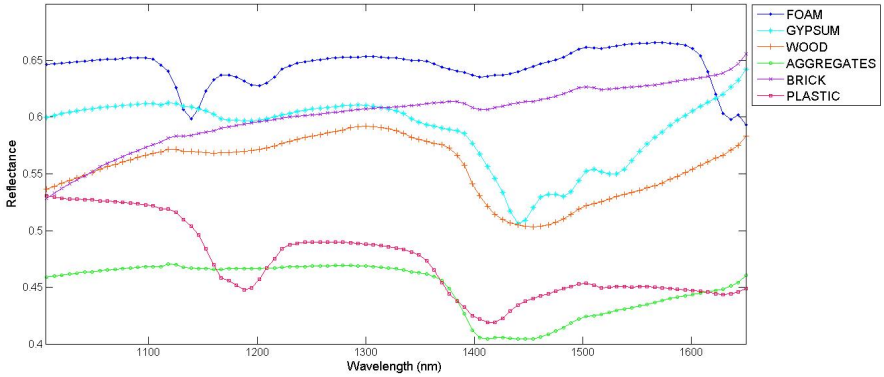

Figure 1 Acquired average spectra of the six different analyzed concrete contaminants

CHEMICAL IMAGING OF PAINTING SURFACES IN THE MID-IR RANGE

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A hyperspectral imaging system (HI90, Bruker Optics), working in the mid-infrared range (1300-900 cm^{-1}) and recently developed for the identification and mapping of gaseous hazardous compounds, has been here applied for the remote investigation of painting materials. The system has been optimized working on laboratory models made of different yellow, white and blue pigments painted with four types of binders (*i.e.*, egg, alkyd, acrylic and vinyl). Two large paintings by Alberto Burri, *Sestante 10* and *Bianco 1952* have been investigated *in situ* through the HI90 system, imaging the distribution of inorganic materials and binding media. In particular, the distribution of different extenders (kaolin, $BaSO_4$, $CaSO_4$) mixed with the various silica-based pigments and two different binders (acrylic and vinyl) has been highlighted. The brightness temperature spectra collected by the HI90 system have been also compared with reflection point infrared spectra acquired by a the conventional portable FTIR spectrophotometer revealing a good spectral quality of hypercube data produced by the imaging system. This comparison permitted also to evaluate the spectral response and assignment from the reduced spectra range available by the HI90 imaging (1300-900 cm^{-1}), validating the reliability of the obtained chemical images. This study clearly highlights the high potential of the hyperspectral imaging in the mid-IR and opens up new perspectives in the application of chemical mapping and imaging methods for the study of painting surfaces.

HYPERSPECTRAL IMAGING APPLIED TO DIAGNOSTICS AND CONSERVATION: A METHODOLOGICAL APPROACH FOR PICTORIAL LAYER CHARACTERIZATION

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The use of Hyper-Spectral Imaging (HSI) as a diagnostic tool in the field of cultural heritage is of great interest presenting high potentialities, being non-destructive, non-invasive and portable. The obtained 2D information (i.e. one spectrum for each pixel of the target image) can be thus used to characterize, map and monitor the materials constituting and artefacts of cultural interest. Such non-invasive diagnostic technique can be applied by mobile devices directly in situ. The adoption of HSI techniques allows the acquisition of the spectra without a direct physical contact and/or sampling of material objects under investigations. This research is addressed to the development of a methodology to classify different pigments through HSI. Reference samples were prepared using 6 pigments (i.e. red ocher, yellow ocher, yellow chrome, blue mineral, cobalt green, and malachite) mixed with four binders (i.e arabic gum, gouache, egg tempera and oil). The obtained products were then applied on two different supports (i.e. canvas and wood) in order to obtain different combinations of pigment/support. The spectral changes of each pigment, according to the different supports, were preliminary evaluated with reference to the spectrum of the original powder pigment. The study was then addressed to verify, according to the information acquired in the different wavelength regions, the correlation existing between collected spectral signatures and sample characteristics related to the different utilized pigments, binders and supports. The resulting reference samples were acquired by HSI in the SWIR (1000-2500 nm) wavelength range. Data were processed adopting a chemometric approach based on the PLS_Toolbox (Version 7.8, Eigenvector Research, Inc.) running inside Matlab® (Version 7.5, The Mathworks, Inc.). The following chemometric methods were applied: ***P**rincipal **C**omponent **A**nalysis* (PCA) for exploratory data approach and ***P**artial **L**east **S**quare- **D**iscriminant **A**nalysis* (PLS-DA) to build classification models. Results showed as it is possible to identify a pigment on the surface of painting using a reference database built according to the collected powder pigments spectra. The developed procedures also allowed to obtain information on the utilized binder and the support.

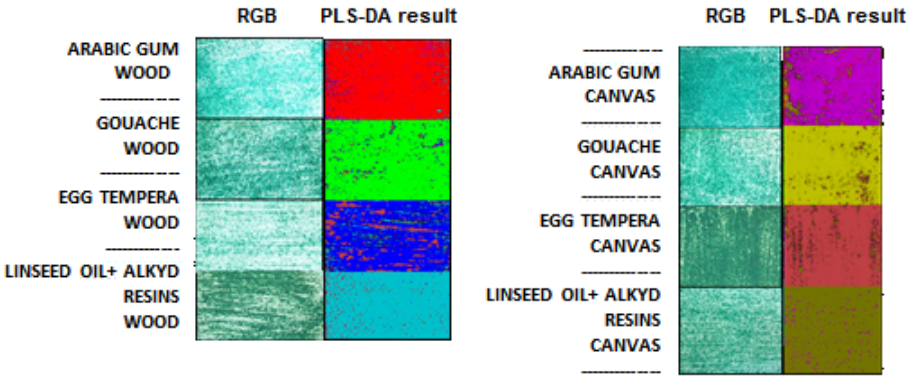

Figure 1 Images representative of most probable class prediction obtained by PLS-DA applied to malachite mixed with 4 different binders on canvas and wood supports.

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HYPERSPECTRAL SYSTEM BASED ON TWO LIQUID CRYSTAL TUNEABLE FILTERS FOR EARLY DETECTION OF CITRUS FRUITS DECAY

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This work presents the development of a hyperspectral system based on two liquid crystal tuneable filters (LCTF) for the acquisition of images of spherical fruits between 400 and 1100 nm and applied to the early detection of decay in citrus fruits. In general, to make inspections covering a wide spectral range it is normal to use more than one system due to the relative small spectral range of individual devices. In the case of using LCTF with different spectral ranges, there is needed to change the filter after each session of acquisition. But it is difficult to ensure that the acquired scene does not vary with each of the filters. Therefore, it is necessary to design a system that allows the filters to be exchanged quickly and without altering the acquired scene, which is proposed here. The two LCTF used are sensitive to the visible range between 400 nm and 720 nm (Varispec VIS07, CRI Inc) and to NIR in the 650 nm to 1100 nm (Varispec NIR07, CRI Inc). The camera used was a CoolSnap ES (Photometrics, USA) which provides high quantum efficiencies in both the visible and the NIR regions but the efficiency of the CCD decreases in a critical way beyond 1030 nm. A Xenoplan 1.4/17mm C-Mount lens (Schneider-Kreuznach, Germany) was used in the acquisition of focused images in the studied ranges. Each fruit was illuminated individually by indirect light from twelve halogen lamps (20 W) inside an aluminium hemispherical diffuser in order to provide good spectral efficiency in the visible and NIR. The lamps were powered by a stabilised power supply (12 V/DC 350 W).

In order to exchange the filters without handling the camera and maintaining unaltered the scene, a guidance system for the filters has been designed. This solution consists in using a tailor-made support that holds the filters inside and moves them by means of a set of runners. The image acquisition is done using a particular application that allows calibrating the system and the exposure times for each band, tuning the filters to particular bands and changing the filters when necessary. To achieve these requirements, the computer is connected to an Arduino platform (http://arduino.cc) that moves a stepper motor connected to the filter holding box trough a belt. This ensures that exactly the same scene is acquired with both filters, since the camera is not touched and therefore there is no risk of changing its position. This system has been used for acquiring and segmenting spectral images of citrus fruits in order to detect fungal infections caused by *P. italicum*, since they provoke very serious postharvest loses around the world, and the external damage caused by this decay is hardly visible to the human eye and even more it is not detected by standard automatic.

A total of 100 mandarins cv. 'Clemenules' were used in the experiments divided in two groups of 50 fruits each, containing sound fruit and fruit inoculated with spores of *P. italicum*. This data set was divided randomly into three subsets, i.e. training set (20%), validation set (20%) and test set (60%). The data set used in the experiments was composed of a total of 80015 pixels that were manually selected from the images of the fruits, and classified into two different classes: pixels belonging to sound skin (class "non-rotten") and pixels belonging to decay lesions (class "rotten"). Segmentation of the hyperspectral images was performed using a method based on artificial neural networks (ANN) that was capable of correctly classifying 98% of pixels as rotten or non-rotten, and 95% of fruit.

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DAILY FRESHNESS DECAY OF MINIMALLY PROCESSED APPLES USING VIS/NIR MULTISPECTRAL IMAGING: PRELIMINARY TESTS

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In recent years, a substantial increase in the consumption of fresh-cut, or minimally processed, fruit and vegetables has been occurred. Changes in human life styles in fact often lead the consumers towards ease of use and healthy products [1]. Monitoring the quality decay of fresh-cut products is necessary to control the freshness level during the entire production chain. Hence fresh-cut fruit and vegetable sector could be greatly helped by new analytical methods that are accurate, rapid and could be integrated into the production chain [2].
The objective of this study was to test vis/NIR multispectral imaging to assess the freshness decay throughout the day (12 hours) of fresh-cut apple slices.
Twelve *Cripps Pink* apples were bought at the same moment at a large retail supermarket, intending to replicate the same experiment every seven days for four weeks on three different apples. The variability of the samples was in this way increased thanks to the natural processes of senescence during the weeks. At every experiment replication, three samples were obtained by cutting a slice from an apple, transversally with respect to the stem-calyx axis. Only half slice portion was treated to simulate a ready-to-eat product, by dipping it for 5 minutes into an aqueous solution of 2% (w/v) L-ascorbic acid, while the other half was left unprocessed as a control portion [3,4]. The apples were monitored every sixty minutes for twelve consecutive hours through the use of an 18-channel imaging system (430-970 nm, VideometerLab, by Videometer A/S, Denmark).
A color correction was performed on each acquisition through a normalization with respect to a multicolor reference acquired together with each sample, in order to take into account the stability of measuring conditions [5]. Each image was segmented by using the k-means algorithm and the apple flesh, i.e. the only part of interest, was separated from the background, the peel and the fruit core. After these pretreatments, all the acquisitions of the day for each slice were put together in a single dataset, unfolded in a two dimensional matrix, and the Principal Component Analysis (PCA) was applied. False color images, colored on the basis of the PC1 scores, were finally recomposed in order to highlight significant differences developed in a single apple slice [5].
The results are encouraging. For all the samples the analysis distinguished between the treated portion of the slice and the unprocessed one, highlighting how this difference is more evident in correspondence of the visible wavelengths. Nevertheless a clear time evolution is shown by almost all the apple slices for both the processed and unprocessed portions. This preliminary study demonstrated the applicability of multispectral imaging as a rapid and non-destructive approach for monitoring the freshness decay throughout the hours of minimally processed apple slices.

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CLASSIFICATION OF *ARABICA* AND *ROBUSTA* COFFEE SAMPLES SUBJECTED TO DIFFERENT TECHNOLOGICAL TREATMENTS USING VARIOUS IMAGE ANALYSIS METHODS

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Coffee varietal differentiation based on NIR spectroscopy has been widely investigated in the last 20 years [1-3]. In this work, we have applied hyperspectral imaging in the NIR range (900-1700 nm) for the classification of *Arabica* and *Robusta* coffee varieties, considering coffee beans subjected to different processing methods, i.e., the so-called *dry method* (to produce *natural coffee*), *wet method* (to produce *washed coffee*) and a somewhat intermediate processing method, referred to as *polishing method* (to produce *polished coffee*).
PCA has been used as an exploratory technique both on the image mean spectra and on the hyperspectrograms obtained from the images. The hyperspectrograms are built by compressing the useful information contained in each hyperspectral image into a signal composed by the frequency distribution curves of quantities calculated by PCA [4]. This procedure allows to compress the information conveyed by the hyperspectral images, maintaining at the same time both spatial- and spectral-related features.
The PCA models obtained showed a clear clustering of *Arabica* and *Robusta* samples, whereas, considering the technological treatment, the *polished* coffee samples are clearly distinguishable from the others, while *natural* and *washed* coffee samples are quite superimposed.
Image mean spectra and hyperspectrograms were then subjected to PLS-DA classification after preprocessing using SNV followed by meancentering or meancentering only. Concerning the discrimination of coffee samples between *Arabica* and *Robusta* categories, the same value of classification efficiency in prediction (EFF_{PRED} = 86.3%) has been obtained considering both the mean spectra and the hyperspectrograms. After forward iPLS-DA variable selection, EFF_{PRED} increased up to 98.6% for models calculated using the mean spectra and up to 100% for the models calculated using the hyperspectrograms.
As for the discrimination of the coffee samples into the three *natural*, *polished* and *washed* processing categories, the PLS-DA models calculated using mean spectra led to EFF_{PRED} values equal to 81.1%, 95.7% and 49.8%, respectively, while the PLS-DA models calculated using hyperspectrograms led to EFF_{PRED} values equal to 94.7%, 100% and 92.4%, respectively. In this case, iPLS-DA variable selection led to an increase of the performances of the model calculated on mean spectra (EFF_{PRED} equal to 82.9%, 98.6% and 86.5%, respectively) and to a decrease of the performances of the model calculated using hyperspectrograms (EFF_{PRED} equal to 82.9%, 89.3% and 86.5%, respectively).

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ANALYSIS OF BAKED SPONGES USING HYPERSPECTRAL IMAGING

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In recent years, hyperspectral imaging systems have reduced in both size and cost resulting in more widespread use in a number of industries and research areas. Food science applications in particular have seen a significant increase. In this presentation, a technique for using hyperspectral imaging to estimate the eating quality of baked sponges with end goal of estimating the shelf life of these products is presented. Existing techniques for this purpose rely on a subjective tasting panel of trained experts to taste sponges on a daily basis to assess their quality. The technique proposed here produces a score for each sponge based on the average observed moisture content. These scores are compared with the results of a tasting panel and it is shown that there is a strong correlation between results. This shows that a robust, repeatable and objective technique for assessing the quality of sponges can be implemented using a hyperspectral imaging system. Furthermore, a classifier is trained to identify moist and dry regions in a sponge image, showing a visualisation of the moisture distribution in a sponge. This gives the bakery more information about the baking process, allowing adjustments to be made and their effects to be immediately analysed and quantified.

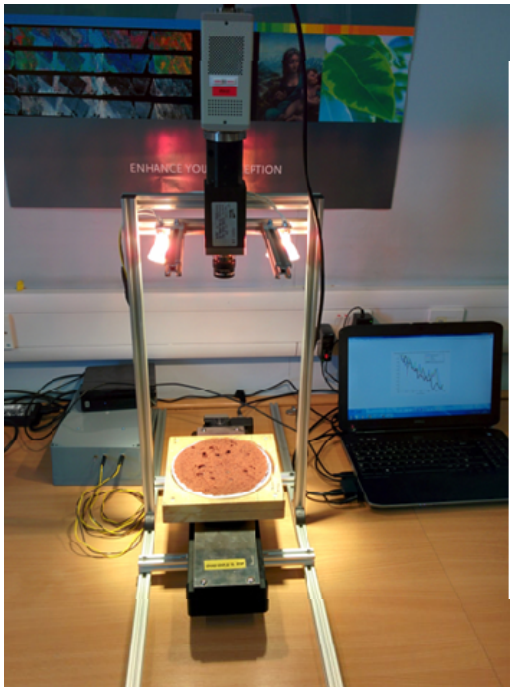

Figure 1 - Imaging Setup

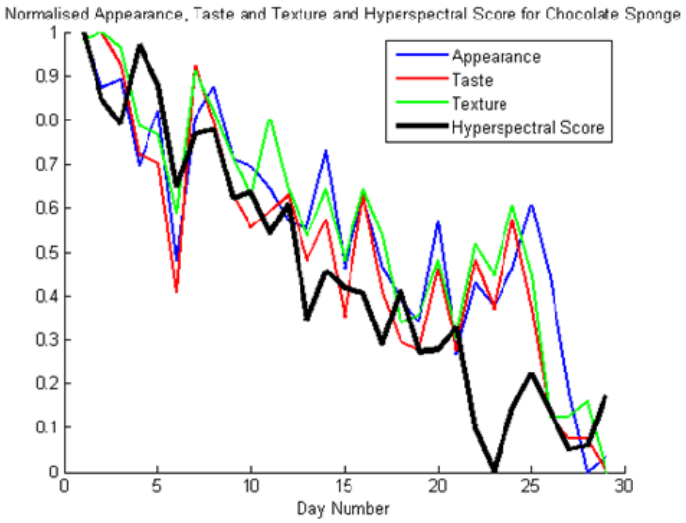

Figure 2 - Normalised Scores

THE USE OF HYPERSPECTRAL IMAGING FOR PREDICTING BEEF EATING QUALITY

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Over the past a few decades, the ability of near infrared reflectance spectroscopy (NIRS) to predict meat quality has been proved by many researchers as an effective and non-destructive approach [1-2]. As an emerging technique, hyperspectral imaging (HSI), which could not only achieve the spectral information but also the spatial information of an object, has aroused the interest of researchers for meat quality analysis. Compared with NIRS with limited scanning area, HSI is able to collect the reflectance spectrum of every single pixel. Thus, for meat quality prediction, a larger effective scanning area can be involved so that the prediction accuracy might be enhanced.

It was found that 3 key factors, which are tenderness, juiciness and flavour, influence the repurchase behaviour of consumers [3]. In this project, slice shear force (SSF) and ultimate pH values were measured as references for these factors. 200 steaks aged for 2 days were imaged by an HSI system (λ = 283 – 863 nm) in the abattoir during two consecutive days. After that, samples were further aged for 5 days and 12 days respectively before SSF and pH measurements. In total, there are 4 quality traits that would be predicted by hyperspectral images in this project, which are SSF aged for 7 days (SSF7), SSF aged for 14 days (SSF14), pH aged for 7 days (pH7) and pH aged for 14 days (pH14).

Support vector machine (SVM) was employed to construct the prediction equation. Considering that SVM is sensitive to the curse of dimensionality, principal component analysis (PCA) was first applied to reduce the dimensionality. Some spectra pre-treatment procedures were also applied to reduce the spectral noise, including singular spectrum analysis (SSA) and transforming reflectance into absorbance. The performance was assessed by the coefficient of determination (R^2) and the ratio of performance deviation (RPD). Results are shown in Table 1. For tenderness prediction, only 1 paper has predicted SSF using NIRS, with an average R^2_{val} of 0.27 [2]. Also, reference [1] gave R^2_{cal} of 0.44 for predicting pH using NIRS, which were both lower than our results. In conclusion, this abstract has demonstrated that HSI is promising in offering additional information for predicting beef quality parameters than NIRS, which could be beneficial to beef industries.

Table 1. Performance for predicting quality traits in beef using HSI.

Trait	Pre-treatment	No. of principal components	Calibration		Validation		
			R^2_{cal}	$RMSE_{cal}$	R^2_{val}	$RMSE_{val}$	RPD_{val}
SSF7	SSA+Absorbance	30	0.35	39.48	0.33	37.81	1.21
SSF14	Reflectance	35	0.35	37.20	0.32	34.97	1.14
pH7	SSA+Absorbance	45	0.83	0.06	0.58	0.10	1.43
pH14	Reflectance	45	1.00	3.85e-4	0.59	0.10	1.42

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HYPERSPECTRAL IMAGING OF DANIO RERIO (ZEBRA FISH): A PRELIMINARY STUDY

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Hyperspectral images provide spatial and spectral information of samples. This characteristic makes the imaging techniques suitable for studying biological tissues because information about the composition and location of biological components in tissues can be obtained. Hyperspectral images have been proposed in several studies to differentiate tissues, e.g., tumor from healthy tissues [1]. Multivariate chemometric techniques are often necessary in hyperspectral image analysis because they are capable to handle and interpret the massive information acquired from raw spectral signals. Among the different chemometric techniques, multivariate curve resolution-alternating least squares (MCR-ALS) [2] is often used to analyze hyperspectral images because it can provide the pure spectra (signatures) and distribution maps of compounds in a sample from the sole raw image measurement.

The study presented is the initial stage of a work, the ultimate objective of which is interpreting the possible biological response of *Danio rerio* to the exposure to some environmental pollutants using Raman and Infrared (IR) hyperspectral images combined with chemometric techniques.

In this early stage of the study, we want to know the potential of Raman and IR hyperspectral imaging to analyze *Danio rerio* embryos and tissues. We will optimize firstly sample treatment protocols of the living organisms before tissue samples are analyzed by hyperspectral imaging. In order to achieve a satisfactory image quality of the different tissues with both spectroscopic techniques (Raman and IR), instrumental parameters linked to image acquisition should be optimized. MCR-ALS is proposed to analyze the obtained hyperspectral images. MCR-ALS resolves (unmixes) the raw hyperspectral image into the spectral signatures of the major biological elements of the sample contributing to the measured spectral signal, and gives their distribution maps on the tissue surface. In this first study, we will explore the outcomes of the MCR-ALS method when hyperspectral images from *Danio rerio* embryos and tissues are analyzed [3].

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HYPERSPECTRAL IMAGING FOR THE ASSESSMENT OF QUALITY AND SAFETY OF SALMON AND COD FINFISH

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Fish is a highly perishable commodity. Spoilage is much faster if the fish is not held at chill temperatures. It is therefore important for anyone concerned with quality of fish to be able to measure the amount of deterioration that has occurred since it was caught.
A lack of a rapid and objective method of non-invasively inspecting based on quality and safety attributes of finfish products can be overcome by integrating two conventional optical sensing technologies of computer vision and spectroscopy.
In recent years, hyperspectral imaging (HSI) has gained a wide recognition as a non-destructive and fast quality and safety analysis and assessment method for a wide range of food products.
HSI can simultaneously acquire both spatial and spectral information that is critical to the detection of food safety and evaluation of food quality attributes.
The aim of this study was to identify critical image features representing quality and safety on finfish using HIS and chemometric tools.
To reach this objective, two hyperspectral images systems operating in the visible range and the near infrared range were applied for non-invasive determination of seven parameters (Total Viable Count or TVC, Total Volatiles Bases of Nitrogen or N-TVB, colour, fat content, Water Holding Capacity or WHC, K-index and presence/absence of nematodes) of two species (*Salmo Salar*, and *Gadus Morhua spp.)*
An amount of 100 cods and 100 farmed salmons (both gutted and filleted) were analysed using two HSI systems (Vis and NIR range) before being submitted to reference analysis.
A spectral image of each fillet in the Vis range was acquired using a pushbroom line-scanning HSI instrument (DV Optics Ltd, Padua, Italy) within the wavelength range of 445-945 nm. The NIR hyperspectral system used comprised a SUI Goodrich SU320M-1.7RT InGaAs camera (Sensors Unlimited, Inc., Princeton, NJ, USA) and a spectrograph (Specim ImSpector N17E; Spectral Imaging Ltd., Oulu,Finland) covering the spectral range between 900 and 1700 nm.
All the statistical analysis was conducted using ENVI 4.6 (research System, Inc., USA) and programs developed in the Matlab R2008b software (The Mathworks, Inc., Natick, MA, USA). PLS and MIA Toolboxes (Eigenvector Technologies, Manson, Washington, USA) were applied to derive the image process of hyperspectral images.
Linear and nonlinear methods for regression and classification like Support Vector Machine (SVM) algorithm [1] and PLS [2] were tested and performance was evaluated in terms of Root Mean Square Error of Calibration (RMSEC) in the calibration process, and Root Mean Square Error of Cross Validation (RMSECV) and coefficient of determination of cross validation (R^2 CV) in the validation process.
Genetic algorithms (GA) [3] were conducted to identify the most important wavelengths/variables that had the greatest influence on predictions throughout the whole wavelength range.
Results obtained confirm the suitability of Vis and NIR HSI approach for salmon and cod freshness prediction.

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NIR HYPERSPECTRAL IMAGING AND SPECTRAL BAND CHARACTERISATION FOR AGRICULTURAL PRODUCTS CONTROL

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The scope of this work is to present a procedure for the treatment of spectral data from Near Infrared Hyperspectral Imaging (NIR-HSI) based on the intensity under a specific wavelength band area. The overall objective is to directly use the chemical information included in the spectra as an alternative to chemometric discrimination tools. By omitting spectral regions the goal is to avoid forcing the analysis to model irrelevant information as noisy, non-linear (flat) or non-informative areas. The specific band is selected based on the loadings obtained from PCA models constructed on reference libraries of the product(s) to be characterized as well as the chemical knowledge of the spectra. Then the area under this band is calculated and used as a monitoring parameter. By fixing thresholds defined by the reference libraries, this specific bands intensity should permit the characterization of a certain product and should allow new spectral types to be flagged, such as levels of fat/protein exceeding a certain value or samples containing an impurity. This technique has several advantages as the interpretation is based on the direct assessment of the chemical molecules which are linked to that specific band. Moreover, as no chemometric discriminant models are constructed, the problem to attempt to model noise and non-linearity disappears.

This methodology has been mainly oriented to quality control and to the detection of adulteration in agricultural products. The method has allowed to follow-up key parameters of products such as mapping of fat content in food and feed products, as well as the detection of modified products. In addition, a comparison with chemometric tools have indicated that the method provided accurate results for the detection of adulterated food products and has thus a certain potential for industrial applications in routine analysis [1].

In this work, real case applications will be presented to characterise products from both the feed and food industries. The feed applications will illustrate the implementation of the method for the quality control of final products while food applications will be oriented towards the detection of adulteration in the raw products prior to processing.

Keywords: Hyperspectral imaging; band characterization; chemometrics; quality control.

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IDENTIFICATION OF COCCIDIOSTATS IN FEED ADDITIVES BY MEANS OF FT-NIR HYPERSPECTRAL IMAGE ANALYSIS

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Coccidiostats are worldwide used drugs for the treatment of coccidiosis. Over twenty coccidiostat compounds are known, however, only eleven compounds have been authorised by the European Union under Regulation (EC) No 1831/2003 for treating and preventing diseases in farm animals. Coccidiostats can be in a wide range 0.5-25 % among the ingredients of the feed additives and up to two coccidiostats can be mixed in the formulation, having up to 40 % of active substance in some of the formulations. The FT-NIR image of different feed additives was captured (6200-4000 cm^{-1}, resolution 4 cm^{-1}, 32 scan/pixel) and analysed by means of PCA and MCR in order to identify the active substances present in the commercial products. Maxiban, rich in Narasin and Nicarbazin at a 40 % shows different colour particles in the formulation, which could be identified by means of FT-NIR imaging. This is the first step in the identification and determination of coccidiostats in Feed Additives.

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ON FIELD SPECTROSCOPY INTEGRATED TECHNIQUES FOR BIOTIC AND ABIOTIC STRESSES MONITORING AND MAPPING IN PRECISION AGRICULTURE

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Since the prehistory the agriculture plays a crucial role in satisfying the basic needs of all human beings and also today is the most important enterprise and world's largest industry. Although the agricultural productivity is mainly dependent on climate , droughts, floods, extreme temperatures and shifting rain patterns many others local factors such as lack of nutrients or water, soil deficiencies, mildews or insects attacks may have a strong impact in reducing the expected harvest. In general the industrial agriculture aims at increasing the harvest, minimizing at same time the costs and improving the quality of the products while the modern precision agriculture relies on site-specific management strategy to maximize yield and profit while reducing environmental impacts such as over-fertilization and the broad applications of pesticides. These are the main objective of the precision agriculture which consists of an optimized new farming management concept based on effective observing and measuring techniques for responding to inter and intra-field variability in crops, by effectively tuning inputs in terms of nutrients , water, fertilizers and pesticides depending on the specific spatial and time dependent plant needs. This approach requires precise measuring and mapping capabilities at field level of key data at a sufficient level of accuracy depending on the availability of equipment that must be also operated at a cost-effective way. In this context the hyperspectral reflectance and active fluorescence detection variations related to chlorophyll and pigments (i.e. carotenoids) plant content [1], will be tested as stress indicators through field surveys and laboratory analyses for rapid assessments, non-destructive and operative methods. These georeferenced field measurements may provide also effective point calibration for multi/hyperspectral imaging remote sensing technologies applications. In this perspective, according to the precision agriculture requirements, our final goal is also to obtain enough accurate and repetitive remote detection and mapping of plant damages at early stages or stresses arising from the above cited factors, by exploiting also the most recent data processing methods, sensors and platforms (satellite, airborne, APR/UAV,..) [2] available at different levels.

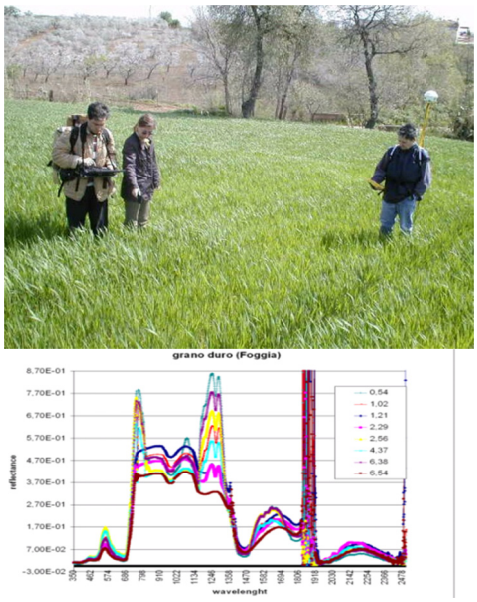

Figure 1 Acquisition of crop georefrenced hyperspectral reflectance signatures over soils at diffent levels of salinization.

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FROM LABORATORY TO FIELD SCALE HYPERSPECTRAL IMAGERY: PLATFORM SETUP AND APPLICATIONS

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Recent developments in hardware and software have increased the possibilities and reduced the costs of hyperspectral proximal sensing. As a non-destructive analytical method, the hyperspectral analysis is suitable to be used for several applications such as vegetation monitoring, control of the water trophic level, separation of different materials, study of soil degradation, asphalt characterization and cultural heritage studies.
Three systems for hyperspectral sensing have been designed and setup at the Laboratory of Hydraulics of DICEA-Sapienza University of Rome, one based on the use of one Spectroradiometer, the other one on two spectrometers, the last one on tunable interference filters. The system based on the spectroradiometer allows sampling the spectral interval 350-2500 nm with 1 nm spectral resolution. This instrument allows detecting physical and chemical properties of materials at a very detailed level. In the case of road infrastructure, the quantification of the relationship among reflectance values and specific road surface characteristics is still an open question. Field data and preliminary laboratory experiments confirm that spectroradiometric data can be successfully used to check and quantify the aggregate exposure to the atmosphere caused by vehicle transit or natural bitumen worsening.
The systems based on the use of spectrometers and interference filters allow sampling the 400–1800 nm spectral range with a spectral resolution lower than 10 nm. The systems have the following unique characteristics: low cost compared to other systems available on the market; high spectral resolution; high spatial and temporal resolution; versatility being suitable for surveys at both laboratory and field scale, easy portability, being the systems engineered so that they can be transported by ultralight airplanes and drones.
A laboratory scale intensive testing has allowed demonstrating the applicability of the two-spectrometer apparatus in the field of material recycling. A rapid and reliable identification of PET from PVC has been achieved by using a simple two near-infrared wavelength operator coupled to a ratio analysis of reflectance spectra. This resulted in 100 % classification accuracy. A sensor based on this identification method appears inexpensive to build and provides the necessary speed and performance required by the recycling industry. The same two-spectrometer apparatus and an original algorithm for automatically combining multiple, overlapping images of a scene to form a single composition, have been employed in a proximal sensing field campaign conducted in San Teodoro (Olbia-Tempio—Sardinia). Mapping allowed for the identification of objects within the acquired image and agreed well with ground-truth measurements.
The system with interference filters was employed during a proximal sensing field survey conducted in the valley of the Sacco river (Latium, Italy). For proximal sensing field surveys with the equipment mounted on a fixed stand, the platform with tunable filters is preferred. This study is part of a larger project dedicated to the recovery of that area, subject in the last few years to a number of alarming cases of pollution (Environmental Quality & Territorial Protection–Sapienza JointLabs). The hyperspectral analysis was employed to detect the environmental status of the region crossed by the river. This was achieved by analyzing a number of spectral indices and waveband combinations derived from the spectral response of White Willow (*Salix Alba*) tree samples located upstream and downstream of an industrial area affected by contamination. A companion lab-scale study on vegetation subject to different types of stress (contamination with herbicides and pesticides, lack of water) was conducted with the same platform to validate the

methodology at a smaller scale (results not shown). Data acquired is represented as reflectance indices as well as reflectance values. Broadband and narrowband indices based on pigment content and carotenoids *vs.* chlorophyll content suggest tree samples located upstream of the contaminated area are 'healthier' than those downstream.

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INSPECTION OF LOG QUALITY BY HYPERSPECTRAL IMAGING

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Wood quality is a key issue for an efficient utilization of harvested logs. The accurate assessment of wood quality and detection of any wood deficiencies at an early stage of the forest-wood processing chain is a highly relevant step for a sustainable organization of the following transformation stream. Hyperspectral imaging (HSI) has been recognized as a powerful technique for inspecting organic matter, including wood, and may provide usable info regarding wood quality in a spatially resolved manner.

A wooden disc of a ~80 year old Norway spruce (*Picea abies (L.) Karst*) was investigated in this pilot study by both, hyperspectral imaging and FT-NIR spectroscopy. The surface of the disk included portions of rough structure, and fairly visible fungal infestation. The sample was evaluated in two moisture conditions including dry and partly wet conditions.

The equipment used was the HeadWall Photonics® push-broom hyperspectral system (Xenics NIR camera: 320*256 matrix, 14 bit A/D, 5 nm resolution, 250 mm Y-table gear, stable diffuse 45/0 illumination). Data acquisition was performed with ARGUS software [1] that automatically performs the background removal, treatment of dead pixels and creation of the hypercubes. The spectral calibration was carried out by measurement of standard surface containing rare earth metals. The optimal signal level (white and dark reference) was calibrated by measuring light reflected from the golden surface (white reference) and dark signal (background noise). Spatial calibration and the proper velocity of Y-table were based on the length of the field of view.

Data mining was performed using a custom hyperspectral data processing algorithm "Cubrowser" [2] developed in Matlab®. The software provides several tools suitable for exploration of hyperspectral images and identification of significant wavelengths attributed to wood defects in different regions of interest. It permits pre-processing of spectra, including noise reduction, handling 3D surface topography and roughness, processing of artifacts produced by lightning conditions, Standard Normal Variate normalization, Savitzky-Golay smoothing, first and second derivation, subtraction or linear combination of different wavelengths to enhance the expression of different structures and deficiencies, among others.

FT-NIR spectrometer Equinox 55 (Bruker®) equipped with the fiber optic probe was used for measurements in regions of interest corresponding to hyperspectral images. It allowed the comparison and analysis of transferability of spectral information gathered by both techniques.

It was found that wooden disk areas affected by fungi and/or structural abnormalities could be clearly identified on the dry and wet wood surface by means of hyperspectral imaging at selected wavelengths or by linear wavelength processing. The influence of wood surface roughness was also negligible, indicating the potential of this technique for application on the freshly harvested logs.

Analysis of FT-NIR spectra revealed further details on the woody polymers degradation by fungi. The comparison of the results gathered by HSI and FT-NIR technique showed, that FT-NIR - as a common method to determine wood quality - can be effectively used as a reference method and for calibration of the wood quality grading system. Summarizing, hyperspectral imaging proved to be a promising technique that, when combined with adequate chemometric models and image processing, could significantly support the on-site quality grading of logs.

Several issues have to be addressed in the subsequent studies, including adjustments due to the uneven moisture distribution, excessively rough surfaces, detection of cracks, and identification of soil or machine oil contaminations and different lightning conditions. The combination of spectroscopic analysis with digital image processing techniques will be further explored to improve the system's capability for detection and identification of most relevant wood defects.

Acknowledgement: We would like to dedicate this work to the memory of Dr. Manfred Schwanninger (1963-2013). This research has been conducted within frame of the SLOPE project (FP7- Collaborative Project – 604129) *"Integrated proceSsing and controL systems fOr sustainable forest Production in mountain arEas"*.

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MT. ETNA (ITALY) HYPERSPECTRAL FIELD DATABASE IN SUPPORT OF MULTISPECTRAL AND HYPERSPECTRAL SATELLITE DATA VALIDATION

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Remotely sensed data, acquired by multispectral and hyperspectral satellite sensors for scientific applications including geology, require periodic validation, since the resulting measurements might be affected by sensor performance changes and accuracy of atmospheric correction models. In this frame we have performed a fieldwork acting to collect spectral reflectance to be compared to multispectral Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER) and hyperspectral (EO1-Hyperion) data. In particular, "dark" (lava flows) and bright (snow) surfaces on Mt. Etna have been investigated allowing, in principle, to test the behavior of both sensors. As concern ASTER we have validated the level2 reflectance product (AST_07). As concern EO1-Hyperion we have applied a customized processing chain to transform the raw data in reflectance before proceed to the comparison between field and satellite reflectance. We found a good accordance of percentage errors (min 2% to a max 18%) in agreement with literature [1] in the ASTER SWIR channels. Relevant errors (up 64%) have been found for the ASTER reflectance products in visible channels.
Regarding EO-1 Hyperion better accordance in reflectance field comparison has been found in the VIS range (5% percentage maximum error vs a 30% value in SWIR range).
Furthermore reflectance data set representative different epochs lava flows over Mt Etna has been realized (Fig.1) providing a significant contribution to enrich the spectroscopic knowledge of the Mount Etna volcano.

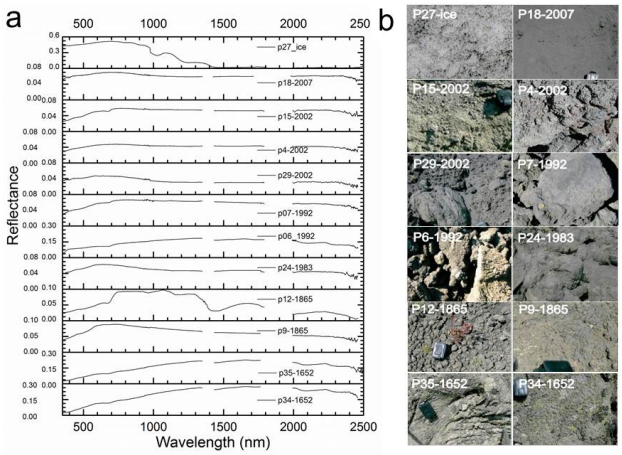

Figure 1 (a) Surface reflectance of different historical Etna lava flows measured with the Fieldspec Spectrometer. The atmospheric absorption bands dominated by noise have been removed. (b)) Pictures of the surfaces are shown. Reflectance of exposed lava and lava plus reddish bushes (1865) and lava and lava with lichens (1992) have been measured representing different endmembers [2]

Acknowledgement: The authors thank D. Pieri, ASTER science Team Member, for the data acquisition planning support and the Jet Propulsion Laboratory, California Institute of Technology for providing ASTER imagery. We further thank the EO1 team for their free data policy, C. Carli for his support in the field measurements.

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NEAR INFRARED IMAGING SPECTROSCOPY ON LITHIC MATERIALS FROM A MESOLITHIC SITE IN NORTHERN SWEDEN.

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In the field of Archaeology, attention to and analysis of lithic materials have strong traditions. Provenance of artefacts and similar questions have been dealt with all through the history of modern Archaeology. The application of NIR spectroscopy to analyse inorganic material in general is steadily growing and characterisation of archaeological materials is of great use in assisting Archaeological interpretations.

This study is a part of the MOBIMA project and the purpose is to show the benefits and applications of using Hyperspectral NIR Imaging spectrometry in the analysis of lithic material on the site level using the Mesolithic Stone Age site at Lillsjön, Ångermanland, Sweden as a case study. Here, the aim is to show the potential of intra site analysis on use of space, and the distribution of different types of lithic materials within the site itself. In figure 1, the spatial distribution of the numbers of items retrieved during the excavation is shown and NIR classification may contribute to a deeper understanding of use of space related to the use of different lithic materials.

Spectral data from Quartz, Quartzite and Flints implements, using Hyperspectral NIR Imaging, enabling large numbers of materials to be scanned in short time frame. Also, because of the heterogeneity of the lithic material, Hyperspectral NIR Imaging will help to selection of optimal spectra for chemometric evaluation.

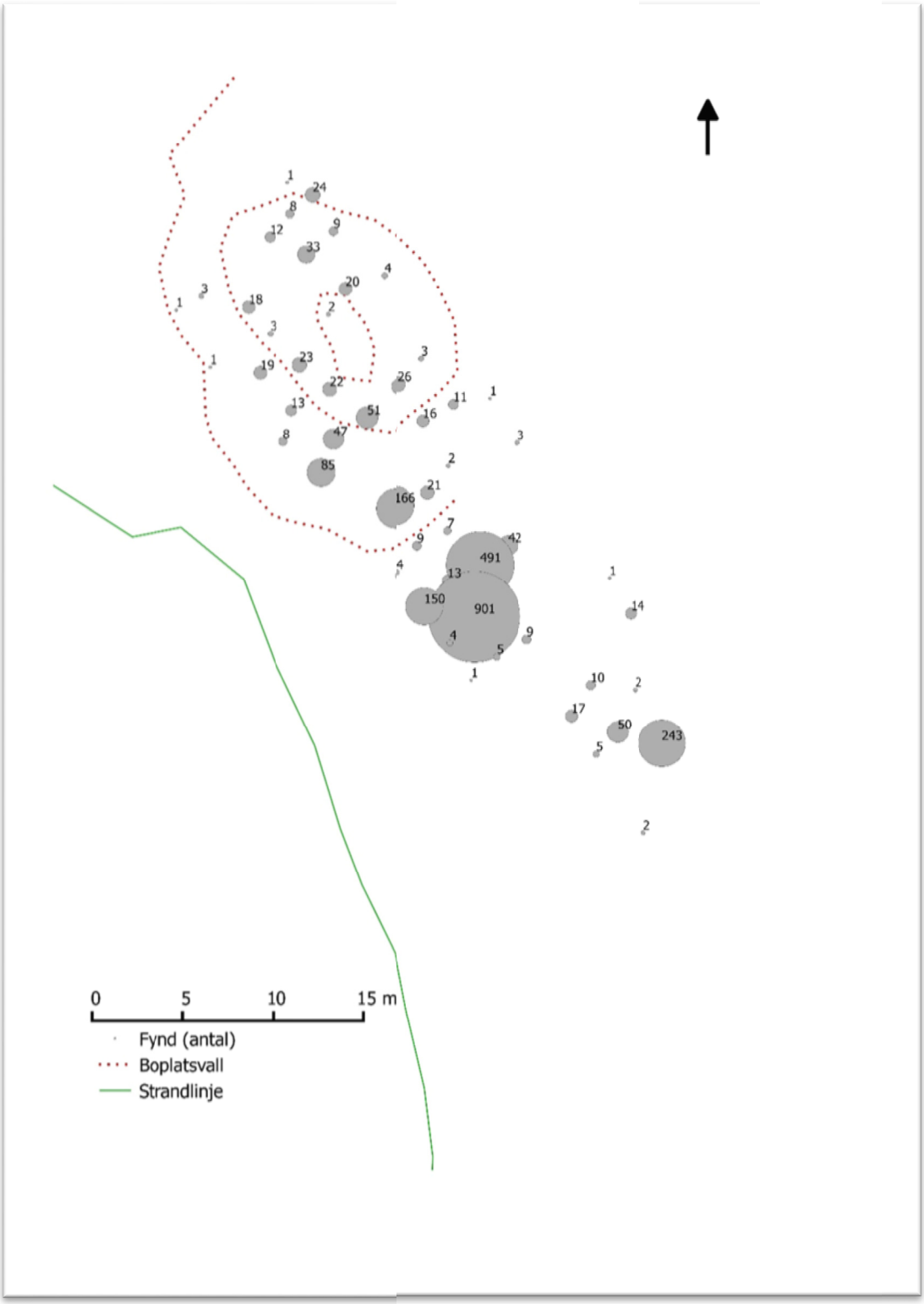

Figure 1 Spatial distribution of lithic finds at the Lillsjön site.

MICRO-RAMAN IMAGING FOR AIR PARTICULATE ANALYSIS

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On March 15, 2014 the city of Lille, following a series of particular weather conditions, caught in a cloud of pollution. This event attracted the interest of the media and the scientific community who, immediately, carried out the air sampling in order to collect the particulate matter contained in it.
Atmospheric aerosols are complex mixtures of natural and anthropogenic particles suspended in the air. With sizes ranging from a few nanometers to tens of micrometers and atmospheric residence times as long as several weeks, aerosols can affect the regional air quality as well as the global climate. Gaining insights into the chemical composition of aerosols at the level of the single particle is necessary to assess their impact on human health and climate change.
However, chemical characterization of individual particles at the micrometer scale is a challenging task. To comply with this objective, confocal Raman micro-spectrometry that combines the spatial resolution of optical microscopy and the molecular analysis capabilities of Raman scattering can acquire molecular information from individual micrometer-sized aerosol particles at ambient laboratory conditions[1].
High spatial resolution Raman confocal microscopy technique can be applied in the analysis of heterogeneous gas-aerosol particle reactions, which are critical in atmospheric chemistry.
Heterogeneous chemistry processes occur in ambient air that can increase dramatically the number of species present in each particle.
Typically the condensation of gaseous pollutants and water vapor on mixed aggregates initiates the formation of complex internal mixtures of several species in one particle.
The aim of this work is to extract simultaneously, from the Raman images, all spectra of pure species and their corresponding spatial distribution within the micrometer scale by using multivariate curve resolution (MCR) technique[2].

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PREPARATION OF HYPERSPECTRAL IMAGE DATA FOR ONLINE MONITORING OF CRYSTALLISATION PROCESS OF PHARMACEUTICAL COMPOUNDS

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In production of high value chemicals as pharmaceuticals, a strict control over the final product attributes like size, purity, morphology, form etc. is required. Traditionally pharmaceuticals are produced in batch mode where it is not possible to fully control process operation and the final product quality varies from batch to batch. Moving from batch processing to continuous operation could offer opportunities for improvement in consistency of the product attributes, however more advance techniques for on-line process monitoring and control are needed.
Here we demonstrated how hyperspectral camera can be used for monitoring of the mixing efficiency of a solute of a pharmaceutical model compound and antisolvent in tubular crystalliser/ The solution is introduced using a turbulent jet. Formation of a solid through antisolvent crystallisation is a very rapid process which could be affected by mixing parameters. A range of images have been acquired to reveal the size and shape of a jet of the injected solution into antisolvent stream as a function of the stream velocity. Two different velocities of solution and antisolvent were tested and spectral distribution of the reactant (Figure 1) as well as final product attributes (Figure 2) was monitored. The jet mixing profile has been found to affects the product with different attributes (PSD and morphology). The hyperspectral camera provides a good technique for on line monitoring a spectral mixing profile and opportunities for control of the process efficiency and final product attributes.
An image processing scheme has been developed that utilizes Principal Component Analysis and morphology to extract the descriptive jet properties from the acquired data.

a) **b)**

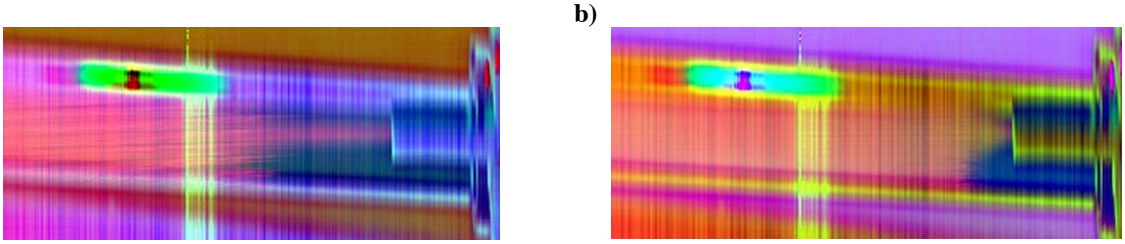

Figure1 HIS image of jet mixing profile for two flow rates, a) 20g/min solution and 80g/min antisolvent (low flow rate), b) 60g/min solution and 240g/min antisolvent (high flow rate).

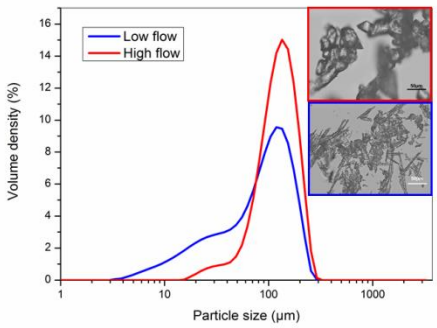

Figure2 Particle size distribution (PSD) and morphology of the final product generated in tubular crystalliser with low and high flow rates.

MULTITECHNIQUE IMAGE ANALYSIS: JOINING RAMAN AND FT-IR BIOLOGICAL TISSUE IMAGES WITH DIFFERENT SPATIAL PROPERTIES.

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The inherent spatial-spectral duality of hyperspectral imaging techniques is highly relevant in different fields of application and, particularly, for the study of biological samples. The simultaneous analysis of images from different platforms recorded on the same sample can be potentially useful to improve tasks, such as the differentiation of healthy and affected biological tissues or the differentiation of subregions within the tissue.

Multiset analysis has been used in the image analysis field to analyze simultaneously sets of images from different samples recorded with the same technique in the same spectral range. This strategy enables coupling images with the same number of spectral channels and variable pixel sizes, geometries and, interestingly, different spatial resolution, because the pixel direction can vary completely among images.

Multiset analysis on images from the same sample collected by different spectroscopic platforms is still seldom used because of the difficulty to acquire joint data sets on the same spatial sample section and the differences of spatial resolution among different spectroscopic techniques. This implies correcting translation/rotation among images and balancing differences in spatial resolution. Translation/rotation differences will be compensated using a non-linear optimization algorithm that can fit shifts in x and y direction and rotation angles among a reference image and a new one to be spatially matched. Differences in spatial resolution will be solved by using an L-shaped data arrangement, in which augmentation in the row direction is performed with matched images with a common pixel mode showing the lowest spatial resolution of the techniques coupled, and in the column direction with the highest resolution image appended with its binned version. This multiset can be resolved (unmixed) with a recent variant of MCR-ALS algorithm for incomplete (irregularly shaped) multisets [2].

This approach is applied to images of the same sample of biological tonsil tissue acquired with different imaging systems (Raman and FT-IR) The outcome expected is an enhancement of the structural description of the image constituents from the complementary information on the two spectroscopic techniques and an improvement of the spatial description associated with the image acquired in the best spatial resolution conditions.

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OVARIAN CYCLE LIPID DYNAMICS REVEALED BY HYPERSPECTRAL DESI-MS IMAGING AND MULTIVARIATE DATA ANALYSIS

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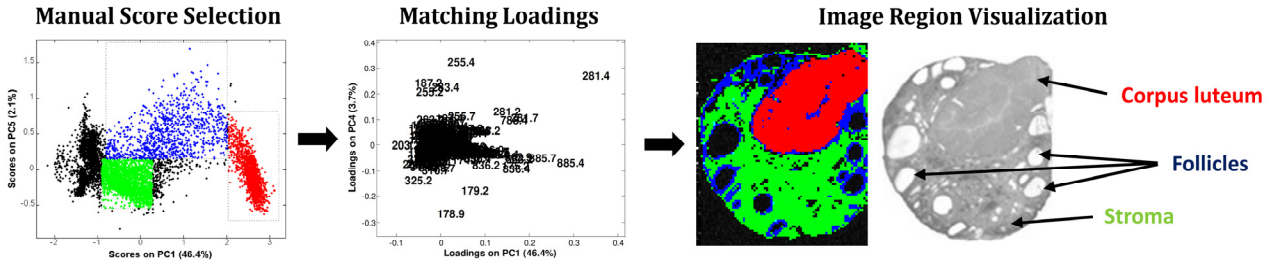
Understanding the role of lipid metabolism in ovarian physiology is crucial for the progression of reproductive biotechnology. The aim in this work was to explore the lipid composition and dynamics of ovarian tissue, specifically the stroma, follicles, and corpora lutea. Desorption electrospray ionization – mass spectrometry (DESI-MS), an ambient ionization technique, was applied in this investigation, acquiring chemical and spatial information simultaneously. A morphologically-friendly solvent, dimethylformamide-acetonitrile (1:1), was used for DESI-MS imaging, which allowed for ovarian lipid characterization and subsequent staining, providing morphological information. Ovaries of mice, swine and bovine were analyzed. Tissue sections (20 µm) were thaw mounted onto glass microscope slides and stored at -80°C until analysis. A linear ion trap mass spectrometer equipped with a custom DESI-MS imaging stage was operated in the negative ion mode (*m/z* 200 to 1000). A 300 x 300 µm pixel size was used in DESI-MS imaging of ovarian tissue. An interactive approach based on principal component analysis (PCA) was used to interpret the chemical and spatial information [1]. A brushing approach was used to visualize the information contained in hyperspectral MS images by automatically connecting regions of PCA score space to pixels of the 2D physical sample. Reduction of the spectral variables (1:3) by a consecutive-window averaging algorithm, normalization with respect to the total ion current (TIC) and mean-centering of variable vectors were performed. Multivariate analyses were carried through in-house Matlab routines.
All morphological structures could be differentiated by PCA and showed similar chemical features independently of the animal species, indicating a conserved lipid constitution. A large variety and abundance of lipids was observed in corpora lutea and follicles, where steroidogenesis is a prominent physiological activity. Smaller differences in the lipid profiles were noted between species, poly-ovulatory and mono-ovulatory species, and reproductive maturation. Additional insight into ovarian physiology was gained with the detection of arachidonic and adrenic acid. The spatial relationship of arachidonic and adrenic acid with the corpora lutea – the former is a known prostaglandin precursor and key signaling molecule in steroidogenesis regulation, and the latter is metabolized in the prostaglandin pathway by the same enzymes – suggests the latter to have also a potential role in steroidogenesis regulation, previously unseen in ovarian physiology. Hyperspectral DESI-MS imaging followed by PCA proved efficient in relating and interpreting the chemical and morphological features. This methodology can by further applied to unravel complex ovarian-related physiological mechanisms and to other physiological and physiopathological models.



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INFRARED THERMAL IMAGING FOR USE IN RESTORATION OF DEFACED SERIAL NUMBERS

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Stamped serial numbers in automotive engines and firearms provide a means of identification; however, they can be obliterated or obscured for criminal activities [1]. Although these can in some instances be restored by chemical etching techniques [2], the discovery of a nondestructive technique to carry out the restoration is highly desirable. Infrared thermal imaging of metal surfaces provides thermal images sensitive to the local differences in thermal conductivity in regions of plastic strain which exist below the defaced serial number [3]. These thermal differences may not be visible from the raw thermal images and further enhancement is needed to pick up these subtle variations. In this study, this enhancement will be executed using multivariate image analysis. Multivariate image analysis (MIA) has become a powerful approach in several fields of endeavor for image processing [4]. Typically, the process involves the use of three dimensional (3D) data sets. Two dimensions are the spatial distances (pixels) and the third can be time, wavelength (for hyperspectral images), or another variable. For this work, thermal images are measured at selected time intervals and stacked to create a 3D hypercube. The MIA procedure involves unfolding the thermal images and carrying out standard principal component analysis to obtain score images and score plots. These results help to reveal the subtle differences in the thermal conductivity in regions of interest (zones of plastic strain) which is further enhanced by segmentation and localization techniques to restore the defaced serial numbers. Presented are results of our analyses to date.

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CHEMOMETRICS REVISITED: HYPERSPECTRAL IMAGING AS A TOOL FOR INVESTIGATING DISTRIBUTIONAL HYPOTHESES

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Among the various advantages of hyperspectral imaging techniques, there is the possibility of recording a very high number of spectra on the systems under exam. This means that, differently than what happens in many real world applications of bulk spectroscopic techniques, where the number of samples is the limiting factor, HSI can provide reliable non-parametric information about the probability distribution of some statistics of interest.
Based on this consideration, the main idea behind the present work is *not to ask (only) what chemometrics can do for hyperspectral imaging, but also what hyperspectral imaging could do for chemometrics*. Accordingly, some distributional hypotheses currently used in chemometric model building (especially in the field of classification techniques) have been tested by means of HSI. It has then been possible to evaluate, e.g., how well grounded the different criteria to define the model space in SIMCA [1,2] are and which one is the closest to the experimental truth.

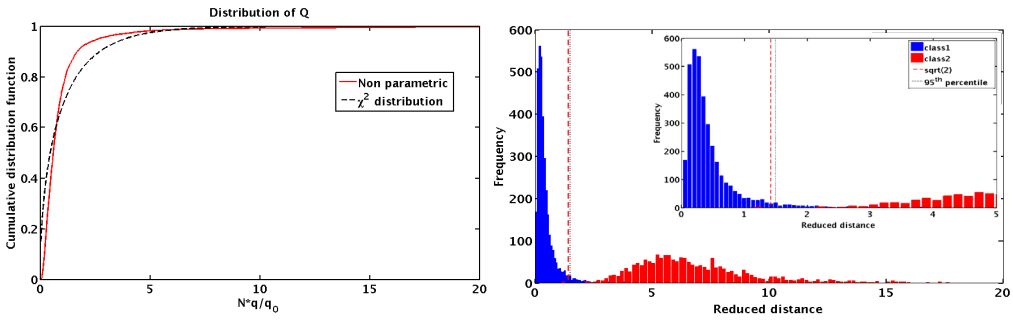

Figure 1 Comparison between HSI data-driven distributions for SIMCA model space criteria and commonly adopted hypotheses and thresholds. (left) Distribution of Q statistics; (right) Distribution of distance to the class model.

Dedication: This communication is dedicated to the memory of Jimi, who was the first inspirer of the work and with whom I've discussed the topic many times.

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